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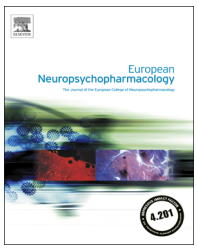

Potentiation of neurite outgrowth by brexpiprazole, a novel serotonin-dopamine activity modulator: A role for serotonin 5-HT_{1A} and 5-HT_{2A} receptors

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5-HT_{2A} receptor

Abstract
Brexpiprazole, a novel atypical antipsychotic drug, is currently being tested in clinical trials for treatment of psychiatric disorders, such as schizophrenia and major depressive disorder. The drug is known to act through a combination of partial agonistic activity at 5-hydroxytryptamine (5-HT)$_{1A}$, and dopamine D_2 receptors, and antagonistic activity at 5-HT_{2A} receptors. Accumulating evidence suggests that antipsychotic drugs act by promoting neurite outgrowth. In this study, we examined whether brexpiprazole affected neurite outgrowth in cell culture. We found that brexpiprazole significantly potentiated nerve growth factor (NGF)-induced neurite outgrowth in PC12 cells, in a concentration dependent manner. The selective 5-HT_{1A} receptor antagonist, WAY-100,635, was able to block the effects of brexpiprazole on neurite outgrowth, unlike the selective dopamine D_2 receptor antagonist, raclopride. Furthermore, the selective 5-HT_{2A} receptor antagonist M100907, but not DOI (5-HT_{2A} receptor agonist), significantly potentiated NGF-induced neurite outgrowth. Moreover, xestospongin C and 2-aminoethoxydiphenyl borate (2-APB), both specific inhibitors of inositol 1,4,5-triphosphate (IP_3) receptors, significantly blocked the effects of brexpiprazole. These findings suggest that brexpiprazole-induced neurite outgrowth is mediated through 5-HT_{1A} and 5-HT_{2A} receptors, and subsequent Ca^{2+} signaling via IP_3 receptors.

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1. Introduction

Brexpiprazole, an atypical antipsychotic drug, is currently being tested in clinical trials evaluating the treatment of schizophrenia, agitation associated with Alzheimer's disease and as an adjunct treatment of post-traumatic stress disorder (PTSD) and major depressive disorder (Citrome, 2013). Brexpiprazole is a potent partial agonist at human 5-hydroxytryptamine (5-HT) 5-HT_{1A} (Ki=0.12 nM) and dopamine D_{2L} (Ki=0.3 nM) receptors, and an antagonist at 5-HT_{2A} receptors (Ki=0.47 nM) (Maeda et al., 2014a). It also shows potent antagonist activity at human noradrenergic α_{1B} (Ki=0.17 nM) and α_{2C} receptors (Ki=0.59 nM) (Maeda et al., 2014a). Furthermore, this drug displays moderate affinity for human D_3, 5-HT_{2B} and 5-HT_7 receptors, as well as α_{1A}, and α_{1D} adrenergic receptors (Maeda et al., 2014a).

Strong evidence suggests that at the cellular level, neuronal plasticity demonstrated by neurite outgrowth and neuroprotection, underlie the therapeutic effects of atypical antipsychotic drugs (Lu and Dwyer, 2005; Williams and Dwyer, 2009; Lieberman et al., 2008; Molteni et al., 2009). PC12 cells, a cell line derived from a pheochromocytoma of the rat adrenal medulla, constitute a recognized model system for nerve growth factor (NGF)-induced neurite outgrowth (Nishimura et al., 2008; Ishima et al., 2008, 2012; Minase et al., 2010; Hashimoto and Ishima, 2010, 2011; Itoh et al., 2011; Ishima and Hashimoto, 2012). Recently, we reported that aripiprazole, an antipsychotic drug, could potentiate NGF-induced neurite outgrowth in PC12 cells, most likely through interaction with 5-HT_{1A} receptors (Ishima et al., 2012).

The primary purpose of this study was to determine whether brexpiprazole could promote NGF-induced neurite outgrowth in PC12 cells. Next, we examined the role of 5-HT_{1A}, 5-HT_{2A} and dopamine D_2 receptors in the mechanistic action of brexpiprazole. We also analyzed the role of intracellular Ca^{2+} and the endoplasmic reticulum (ER) protein, inositol 1,4,5-triphosphate (IP_3) receptor, on brexpiprazole mediated potentiation of NGF-induced neurite outgrowth. This was deemed relevant since Ca^{2+} signaling via IP_3 receptors is crucial to NGF-induced neurite outgrowth (Nishimura et al., 2008; Ishima et al., 2008, 2012; Minase et al., 2010; Hashimoto and Ishima, 2010, 2011; Itoh et al., 2011; Ishima and Hashimoto, 2012). Finally, we examined whether brexpiprazole increased the heat shock protein, Hsp90α, since regulation of Hsp90α by aripiprazole appeared to potentiate the process of neurite outgrowth (Ishima et al., 2012).

2. Experimental procedures

2.1. Drugs

Drugs were obtained from the following sources: brexpiprazole and aripiprazole (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan); xestospongin C and M100,907 (volinanserin) (Wako Pure Chemicals Inc., Tokyo, Japan); WAY-100,635, fluoxetine hydrochloride, paroxetine hydrocloride and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminpprapane (DOI) hydrochloride (Sigma-Aldrich, St Louis, MO, USA); nerve growth factor (NGF) (Alomone Labs Ltd., Jerusalem, Israel); and raclopride (Tocris Bioscience, Bristol, UK); 2-aminoethoxydiphenyl borate (2-APB) (Calbiochem-Novabiochem, San Diego, CA, USA). All other drugs were purchased from commercial sources. All drugs (10 mM) were dissolved in dimethyl sulfoxide (DMSO), and then were diluted in the medium. The final concentration of DMSO did not affect the cell viability in PC12 cells.

2.2. Cell culture and quantification of neurite outgrowth

PC12 cells (RIKEN Cell Bank, Tsukuba, Japan) were cultured at 37 °C, 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% heat-inactivated fetal bovine serum (FBS), 10% heat-inactivated horse serum, and 1% penicillin-streptomycin. Medium was changed two to three times a week. PC12 cells were plated onto 24-well tissue culture plates coated with poly-D-lysine/laminin. Cells were plated at relatively low density (0.25×10^4 cells/cm^2) in DMEM medium containing 0.5% FBS, 1% penicillin-streptomycin. Medium containing a minimal level of serum (0.5% FBS) was used as previously reported (Nishimura et al., 2008; Ishima et al., 2008, 2012; Minase et al., 2010; Hashimoto and Ishima, 2010, 2011; Itoh et al., 2011; Ishima and Hashimoto, 2012). Previously, we examined the optimal concentration of NGF needed to induce neurite outgrowth in PC12 cells, and found that NGF (2.5, 5, 10, 20, 40 ng/ml) increased the number of cells with neurite outgrowth in a concentration-dependent manner (Nishimura et al., 2008). In this study, 2.5 ng/ml of NGF was used to study the potentiating effects of brexpiprazole on neurite outgrowth. Twenty-four hours after plating, the medium was replaced with DMEM medium containing 0.5% FBS and 1% penicillin-streptomycin with NGF (2.5 ng/ml), with or without brexpiprazole (0.001, 0.01, 0.1 or 1.0 μM), WAY-100,635 (5-HT_{1A} receptor antagonist; 10 μM), raclopride (dopamine D_2 receptor antagonist; 10 μM), DOI (5-HT_{2A} receptor agonist; 0.1, 1.0 or 10 μM), M100,907 (5-HT_{2A} receptor antagonist; 0.1, 1.0 or 10 μM), xestospongin C (IP_3 receptor antagonist; 1.0 μM), 2-APB (IP_3 receptor antagonist; 100 μM), fluoxetine (5-HT transporter inhibitor: 1.0 μM), or paroxetine (5-HT transporter inhibitor: 1.0 μM).

Four days after incubation with NGF (2.5 ng/ml) with or without specified drugs, morphometric analysis was performed on digitized images of live cells taken under phase-contrast illumination, with an inverted microscope linked to a camera. Images of three fields per well were taken, with an average of 100 cells per field. Differentiated cells were counted by visual examination of the field; only cells that had at least one neurite with a length equal to the cell body diameter were counted, and were then expressed as a percentage of the total cells in the field. Counting was performed in a blinded manner.

2.3. Western blot analysis of HSP90α

Western blot analysis was performed as reported previously (Ishima et al., 2012). PC12 cells were washed with PBS and lysed in Laemmli lysis buffer. Aliquots (30 μg) of protein were measured using the DC protein assay kit (Bio-Rad, Hercules, CA, USA) and incubated for 5 min at 95 °C, with an equal volume of 125 mM Tris/HCl, pH 6.8, 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol, 4% SDS, and subjected to SDS-PAGE, using 7.5% mini-gels (Mini Protean II; Bio-Rad, Hercules, CA, USA). Proteins were transferred onto PVDF membranes using a Trans Blot Mini Cell (Bio-Rad, Hercules, CA, USA). For immunodetection, the blots were blocked for 1 h in TBST (50 mM Tris/HCl, pH 7.8, 0.13 M NaCl, 0.1% Tween 20) containing 5% nonfat dry milk at RT, followed by incubation with rabbit anti-HSP90α antibody (1:2000, ab2928, Abcam, Cambridge, UK), overnight at 4 °C, in 5% TBST blocking buffer. The blots were washed five times with TBST. Incubation with the secondary antibody (GE Healthcare Bioscience, UK) was performed for 1 h, at RT. After extensive washing, immunoreactivity was detected by ECL Prime Western Blotting Detection reagent (GE Healthcare Bioscience, UK). Images were captured and immunoreactive bands were quantified using a Fuji LAS3000-mini imaging system (Fujifilm, Tokyo, Japan) with Multi Gauge software (Ver.3.0; Fujifilm, Tokyo, Japan). β-actin immunoreactivity was used to monitor equal sample loading.

2.4. Statistical analysis

Data are expressed as means ± standard error of the mean (S.E.M.). Statistical analysis was performed using one-way analysis of variance (ANOVA) or two-way ANOVA. When appropriate, *post-hoc* comparisons were performed using the Bonferroni/Dunn test. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Brexpiprazole potentiated NGF-induced neurite outgrowth in PC12 cells

As shown in Figure 1A, brexpiprazole (1.0 μM) increased the number of cells with neurites in PC12 cells. One-way ANOVA analysis revealed that the number of cells with neurites of six groups was significantly different (F (5, 66) = 16.95, $P < 0.001$). Treatment with brexpiprazole (0.001, 0.01, 0.1 or 1.0 μM) in conjunction with NGF (2.5 ng/ml) increased the number of cells with neurites, in a concentration-dependent manner (Figure 1B). Effect of brexpiprazole (1.0 μM) on neurite outgrowth was similar to that of aripiprazole (1.0 μM) (Figure 1B).

3.2. Role of 5-HT_{1A} receptors in brexpiprazole mediated potentiation of NGF-induced neurite outgrowth

In order to examine the role of 5-HT_{1A} receptor in brexpiprazole's action on neurite outgrowth, we examined the effects of selective 5-HT_{1A} receptor antagonist WAY-100,635. Two-way ANOVA analysis revealed a significant interaction [brexpiprazole × WAY-100,635: F (1, 20) = 11.31, P = 0.003]. Treatment with WAY-100,635 (10 μM) significantly blocked the potentiation of NGF-induced neurite outgrowth by brexpiprazole (1.0 μM) (Figure 2A). In contrast, two-way ANOVA analysis revealed no differences in interaction [brexpiprazole × raclopride: F (1, 20) = 1.260, P = 0.275]. Treatment with raclopride (10 μM) failed to potentiate neurite outgrowth in the presence of NGF and brexpiprazole (Figure 2B). Furthermore, WAY-100,635 (10 μM) alone or raclopride (10 μM) alone did not alter NGF-induced neurite outgrowth in PC12 cells (Figure 2A and B). Collectively, these findings suggest that activation at 5-HT_{1A} receptors, as opposed to dopamine D_2 receptors, may play a role in the mechanisms of brexpiprazole-induced neurite outgrowth.

3.3. Role of 5-HT_{2A} receptors in brexpiprazole mediated potentiation of NGF-induced neurite outgrowth

In order to examine the role of 5-HT_{2A} receptor in brexpiprazole's action on neurite outgrowth, we examined the effects of selective 5-HT_{2A} receptor agonist DOI and 5-HT_{2A} receptor antagonist M100,907. One-way ANOVA analysis of DOI's data revealed no difference [F (3, 44) = 1.253, P = 0.302](Figure 3A). In contrast, one-way ANOVA analysis of M100,907's data revealed a significant effect [F (3, 68) = 14.73, $P < 0.001$]. Treatment with M100,907 (0.1, 1.0, or 10 μM) potentiated NGF-induced neurite outgrowth, in a concentration dependent manner (Figure 3B). Collectively, these findings suggest that antagonism at 5-HT_{2A} receptors may play a role in the mechanisms of brexpiprazole-induced neurite outgrowth.

3.4. The potentiating effect of brexpiprazole on the effects of fluoxetine and paroxetine on NGF-induced neurite outgrowth

We examined whether brexpiprazole could potentiate the effect of the antidepressants (fluoxetine and paroxetine) on neurite outgrowth. One-way ANOVA analysis revealed a significant effect [F (5, 66) = 11.24, $P < 0.001$]. Post-hoc analysis showed that fluoxetine (1.0 μM), but not paroxetine (1.0 μM), significantly potentiated neurite outgrowth, consistent with a previous report (Nishimura et al., 2008). Addition of brexpiprazole (1.0 μM) significantly could potentiate the effects of fluoxetine, but not paroxetine, on neurite outgrowth (Figure 4).

3.5. Role of IP_3 receptors on the potentiation of NGF-induced neurite outgrowth by brexpiprazole

The IP_3 receptors on the ERs are part of the signaling pathway that promotes NGF-induced neurite outgrowth in PC12 cells (Nishimura et al., 2008; Ishima et al., 2008, 2012; Minase et al., 2010; Hashimoto and Ishima, 2010, 2011; Itoh et al., 2011; Ishima and Hashimoto, 2012). To investigate the role of IP_3 receptors in brexpiprazole's action on neurite outgrowth, we examined the effects of xestospongin C (a selective, reversible and membrane-permeable inhibitor of IP_3 receptors) (Gafni et al., 1997) and 2-APB (Maruyama et al., 1997; Ma et al., 2000) on the culture system. Two-way ANOVA analysis revealed significant interactions [brexpiprazole × xestospongin C: F (1, 20) = 12.63, P = 0.002, brexpiprazole × 2-APB: F (1, 20) = 24.48, $P < 0.001$]. Treatment of xestospongin C (1.0 μM) or 2-APB (100 μM) with brexpiprazole (1.0 μM) significantly blocked the potentiation of NGF-induced neurite outgrowth (Figure. 5A and B). Neither xestospongin C (1.0 μM) nor 2-APB (100 μM) in the absence of brexpiprazole promoted NGF-induced neurite outgrowth in PC12 cells (Figure. 5A and B).

3.6. Role of Hsp90α in the potentiation of NGF-induced neurite outgrowth by brexpiprazole

Using a proteomics technique, we recently identified the heat shock protein Hsp90α as being differentially expressed between control PC12 cells treated with NGF (2.5 ng/ml) and those treated with NGF (2.5 ng/ml) and aripiprazole (1.0 μM)(Ishima et al., 2012). Previously, we reported that increased expression of Hsp90α by aripiprazole was involved in the potentiation of NGF-induced neurite outgrowth in PC12 cells (Ishima et al., 2012).

To interrogate the proposed link between brexpiprazole treatment and elevated Hsp90α production on NGF-induced neurite outgrowth, we performed Western blot analysis of Hsp90α. Similar to aripiprazole (1.0 μM), brexpiprazole (1.0 μM) significantly increased Hsp90α protein in PC12 cells (Figure 6).

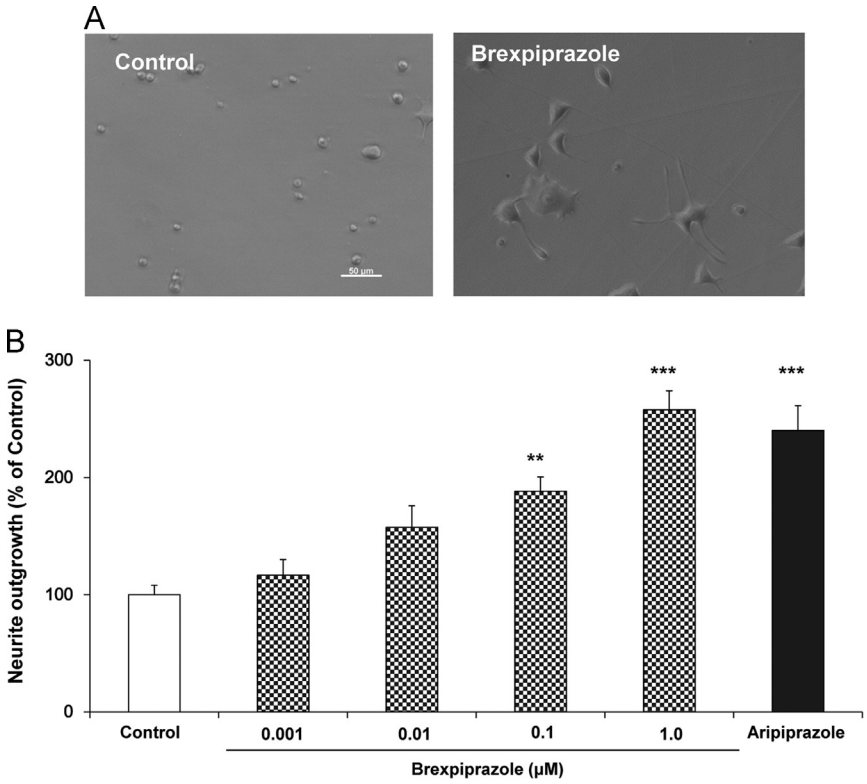

Figure 1 Brexpiprazole potentiated NGF-induced neurite outgrowth in PC12 cells. (A) Representative photomicrographs in PC12 cells. Control: NGF (2.5 ng/ml) alone, Brexpiprazole: NGF (2.5 ng/ml)+brexpiprazole (1.0 μM). Bar=50 μm (B) Effects of brexpiprazole and aripiprazole on NGF-induced neurite outgrowth in PC12 cells. Brexpiprazole (0.001, 0.01, 0.1, or 1.0 μM) and aripiprazole (1.0 μM) potentiated NGF-induced neurite outgrowth in PC12 cells, in a concentration-dependent manner. $^{**}P<0.01$, $^{***}P<0.001$ as compared with Control group. Data show the mean±SEM ($n=12$).

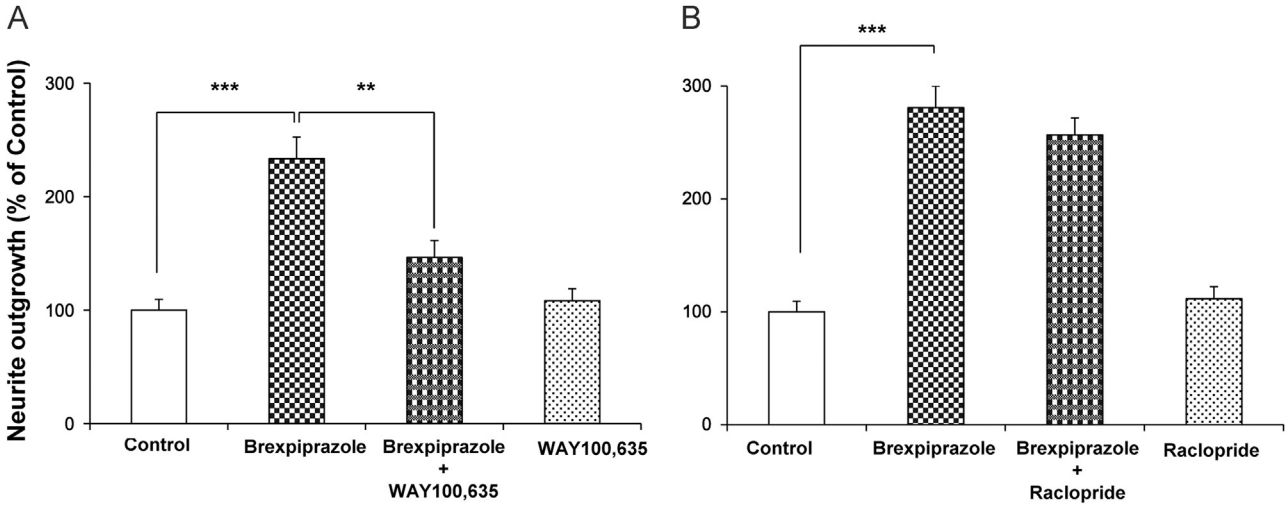

Figure 2 Role of 5-HT_{1A} receptor in the mechanisms of brexpiprazole's potentiation of neurite outgrowth. (A) The selective 5-HT_{1A} receptor antagonist, WAY-100,635 (10 μM), blocked the effects of brexpiprazole. Data show the mean±SEM ($n=6$). (B) The dopamine D_2 receptor antagonist raclopride (10 μM) had no effect of brexpiprazole in NGF-induced neurite outgrowth. $^{**}P<0.01$, $^{***}P<0.001$ as compared with NGF (2.5 ng/ml)+brexpiprazole (1.0 μM) group. The data show the mean±SEM ($n=6$).

4. Discussion

Our study found that brexpiprazole, a novel serotonin-dopamine activity modulator, potentiated NGF-induced neurite outgrowth in PC12 cells, through 5-HT_{1A} receptors and 5-HT_{2A} receptors, and subsequent Ca^{2+} signaling via IP_3 receptors on the ER. Brexpiprazole is currently undergoing testing in clinical trials as a therapy for neuropsychiatric disorders. It is thought to act by partial agonism at 5-HT_{1A}, and dopamine D_2 receptors, and antagonism at 5-HT_{2A} receptors. Brexpiprazole potentiated NGF-induced neurite outgrowth in PC12 cells, and this effect was similar to

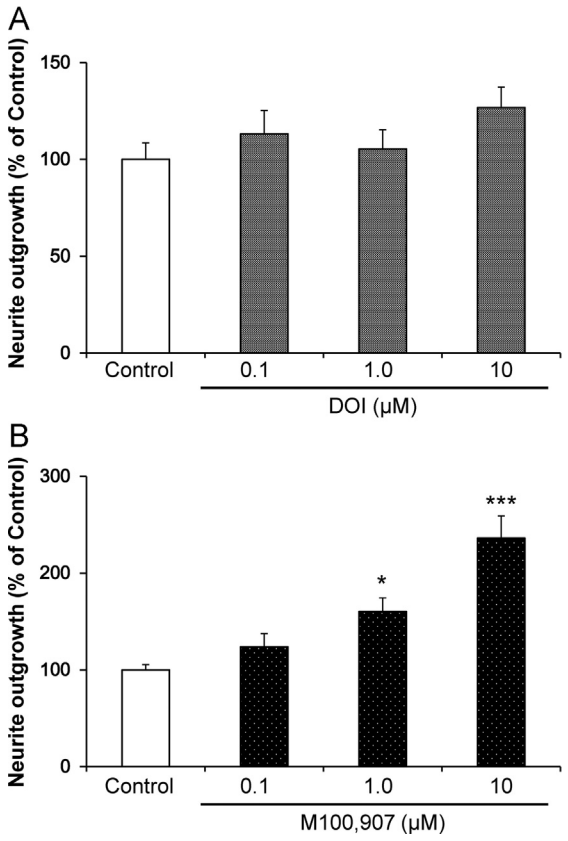

Figure 3 Role of 5-HT_{2A} receptor in the mechanisms of brexpiprazole's potentiation of neurite outgrowth. (A) The selective 5-HT_{2A} receptor agonist DOI (0.1, 1.0, or 10 μM) did not effect NGF-induced neurite outgrowth. Data show the mean ± SEM (n=12). (B) The selective 5-HT_{2A} receptor antagonist M100,907 (0.1, 1.0, or 10 μM) potentiated NGF-induced neurite outgrowth. *P<0.05, ***P<0.001 as compared with NGF (2.5 ng/ml) alone group. The data show the mean ± SEM (n=18).

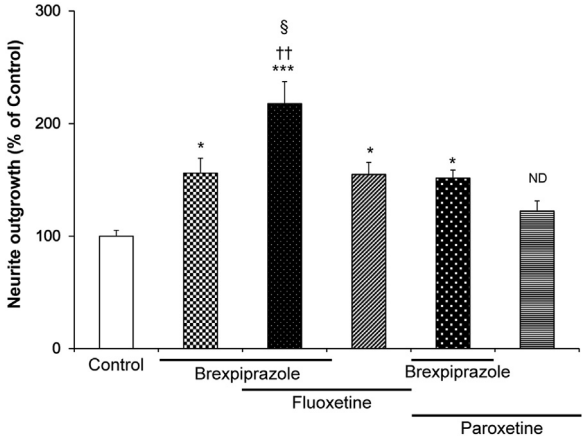

Figure 4 Effect of brexpiprazole on the effects of NGF-induced neurite outgrowth by fluoxetine (1.0 μM) or paroxetine (1.0 μM). Addition of brexpiprazole (1.0 μM) potentiated the effects of fluoxetine, but not paroxetine, on NGF-induced neurite outgrowth. Data show the mean ± SEM (n=12). *P<0.05, ***P<0.001 as compared with control group. ^{+}P<0.05 as comparted with fluoxetine group N.S.: Not significant.

aripiprazole. This potentiation of neurites outgrowth could be partially blocked by the selective 5-HT_{1A} receptor antagonist WAY-100,635, but not the dopamine D_2 receptor antagonist raclopride. Previously, we reported that the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT also potentiated NGF-induced neurite outgrowth in PC12 cells, although to a lesser degree than aripiprazole (Ishima et al., 2012). In this study, we found that the selective 5-HT_{2A} receptor antagonist M100,907 could potentiate NGF-induced neurite outgrowth in PC12 cells, suggesting that 5-HT_{2A} receptor antagonism also may play a role in the mechanisms of action of brexpiprazole. Thus, it would appear that activation of 5-HT_{1A} receptors as well as blockage at 5-HT_{2A} receptors is an integral part of the mechanisms that drive brexpiprazole enhancement of NGF-induced neurite outgrowth.

Previously, we reported that fluoxetine, but not paroxetine, could potentiate NGF-induced neurite outgrowth in PC12 cells, and that the sigma-1 receptor antagonist NE-100 significantly antagonized the effect of fluoxetine (Nishimura et al., 2008). The study suggests that sigma-1 receptor agonism, but not 5-HT transporter inhibition, plays a role in the mechanisms of action of fluoxetine on neurite outgrowth (Nishimura et al., 2008; Hashimoto, 2009, 2013). In this study, we found that fluoxetine, but not paroxetine, could potentiate NGF-induced neurite outgrowth, consistent with a previous report (Nishimura et al., 2008). Interestingly, we found that brexpiprazole significantly could potentiate the effects of fluoxetine (or paroxetine) on neurite outgrowth. Since brexpiprazole has been used as add-on therapy of the current antidepressant therapy, the potentiating effect of brexpiprazole on neurite outgrowth is of great interest.

Very recently, Maeda et al. (2014b) reported that brexpiprazole reversed cognitive impairment in rats after subchronic treatment with the *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP), and that this effect was antagonized by co-treatment with WAY-100,635, a 5-HT_{1A} receptor antagonist. Subsequently, we found that brexpiprazole ameliorated cognitive deficits in mice after repeated dosing with PCP, and that this effect was antagonized by co-treatment with WAY-100,635 (Yoshimi et al., 2014). Very recently, we reported that brexpiprazole improved social recognition deficits in mice after administration of the NMDA receptor antagonist dizocilpine, and that this effect was antagonized by WAY-100,635 (Yoshimi et al., 2015). These findings suggest a role for 5-HT_{1A} receptors in the mechanisms of action of brexpiprazole for PCP (or dizocilpine)-induced cognitive impairment (Maeda et al., 2014b; Yoshimi et al., 2014, 2015). Given that altered 5-HT_{1A} receptor function is implicated in schizophrenia related cognitive impairment (Sumiyoshi et al., 2007; Meltzer and Sumiyoshi, 2008; Hagiwara et al., 2008; Yoshida et al., 2012), activation of this receptor by brexpiprazole may confer a beneficial effect in psychiatric diseases, where patients suffer cognitive deficits. So far these data suggest that the therapeutic actions of brexpiprazole are in part mediated by activation of 5-HT_{1A} receptors.

Inositol 1,4,5-triphosphate is a ubiquitous second messenger responsible for the release of Ca^{2+} from the ER, a tightly controlled process which is critically important for maintaining cellular functions, including cell growth, and neurite outgrowth (Berridge, 1993; Iketani et al., 2009). In this study, we

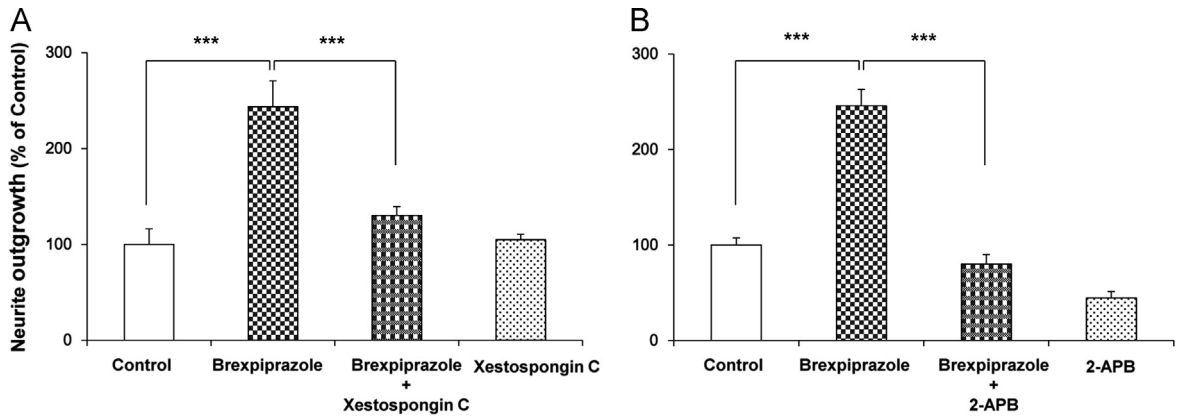

Figure 5 Effects of the IP_3 receptor antagonists on the potentiation of NGF-induced neurite outgrowth by brexpiprazole. (A, B) The potentiating effects of brexpiprazole (1.0 μM) on NGF-induced neurite outgrowth were antagonized by the selective IP_3 receptor antagonists, xestospongin C (1.0 μM) and 2-APB (100 μM). Neither, xestospongin C (1.0 μM) nor 2-APB (100 μM) alone altered NGF-induced neurite outgrowth. Data show the mean ± SEM (n=6). ***P<0.001 as compared with brexpiprazole group.

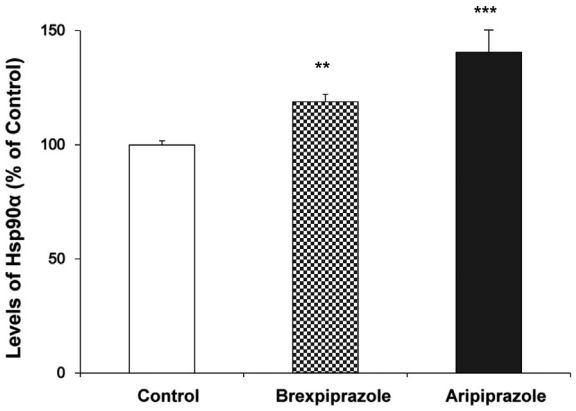

Figure 6 An increase in Hsp90α protein is required for brexpiprazole-induced potentiation of NGF-induced neurite outgrowth. Similar to aripiprazole (1.0 μM), brexpiprazole (1.0 μM) significantly increased Hsp90α protein in PC12 cells. Data show the mean ± SEM (n=8–18). **P<0.01, ***P<0.001 as compared with the control (NGF (2.5 ng/ml) alone) group.

found that IP_3 receptor antagonists, xestospongin C and 2-APB, significantly blocked potentiation of NGF-induced neurite outgrowth by brexpiprazole, highlighting the role of IP_3 receptors and intracellular Ca^{2+} signaling in this process. We already know that several drugs utilize IP_3 receptors to potentiate NGF-induced neurite outgrowth (Nishimura et al., 2008; Ishima et al., 2008, 2012; Minase et al., 2010; Hashimoto and Ishima, 2010, 2011; Itoh et al., 2011; Ishima and Hashimoto, 2012). It is therefore reasonable to conclude that Ca^{2+} signaling via IP_3 receptors in the ER plays an important role in the mechanism of action of brexpiprazole in its potentiation of NGF-induced neurite outgrowth.

Considering the function of the aforementioned signaling molecules in regulating protein synthesis-dependent learning and memory (Costa-Mattioli et al., 2009), brexpiprazole driven changes in these pathways may promote synthesis of new proteins associated with neurite outgrowth. Hsp90 is an essential molecular chaperone, ubiquitously active in many signaling and cellular pathways (Mollapour and Neckers, 2012; Hartson and Matts, 2012; Makhnevych and Houry, 2012). Current data suggest that Hsp90 participates in the assembly of a number of protein complexes (Makhnevych and Houry, 2012), including some required for antipsychotic drug action. We found that the 5-HT_{1A} receptor agonist, 8-OH-DPAT also increased Hsp90α protein levels in PC12 cells, although its effect was less pronounced than that of aripiprazole (Ishima et al., 2012). This places 5-HT_{1A} receptor activation within the cascade leading to increased Hsp90α protein, although the precise mechanisms are unknown. It would appear that brexpiprazole therapy increases Hsp90α protein levels which potentiate NGF-induced neurite outgrowth again, by unknown mechanisms. Future studies need to investigate whether serum levels of Hsp90α are altered in patients with psychiatric disorders and explore whether brexpiprazole may have any effect on those levels. It may be that molecules which increase Hsp90α protein levels confer a therapeutic advantage in psychiatric and neurodegenerative conditions by altering neurite outgrowth.

In conclusion, our results suggest that brexpiprazole potentiates NGF-induced neurite outgrowth in PC12 cells, through activation of 5-HT_{1A} and 5-HT_{2A} receptors and subsequent Ca^{2+} signaling, via IP_3 receptors. Furthermore, it is clear that the increased levels of Hsp90α protein induced by brexpiprazole, also play a role in NGF-induced neurite outgrowth.

Role of the funding source

This study was supported by a research grant from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). The funder had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Dr. Hashimoto designed the study and wrote the protocol. Dr. Ishima performed the experiment of this study. Dr. Ishima and Dr. Hashimoto undertook the statistical analysis. Dr. Futamura, Dr. Ohgi, Ms. Yoshimi and Dr. Kikuchi contributed the reagents/materials/analysis tools. Dr. Hashimoto wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

Dr. Hashimoto has served as a scientific consultant to Astellas and Taisho, and he has also received research support from Abbvie, Dainippon Sumitomo, Otsuka, and Taisho. All other authors declare that they have no conflicts of interest. Dr. Futamura, Dr. Ohgi, Ms. Yoshimi, and Dr. Kikuchi are employer of Otsuka Pharmaceutical Co., Ltd which has developed brexpiprazole and aripiprazole.

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