Possible Role of BDNF-Induced Microglial Intracellular Ca^{2+} Elevation in the Pathophysiology of Neuropsychiatric Disorders

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Abstract: Microglia are intrinsic immune cells that release factors, including proinflammatory cytokines, nitric oxide (NO) and neurotrophins, following activation after disturbance in the brain. Elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) is important for microglial functions, such as the release of cytokines and NO from activated microglia. There is increasing evidence suggesting that pathophysiology of neuropsychiatric disorders is related to the inflammatory responses mediated by microglia. Brain-derived neurotrophic factor (BDNF) is a neurotrophin well known for its roles in the activation of microglia as well as in pathophysiology and/or treatment of neuropsychiatric disorders. We have recently reported that BDNF induces a sustained increase in $[Ca^{2+}]i$ through binding with the truncated TrkB receptor, resulting in activation of the PLC pathway and store-operated calcium entry (SOCE) in rodent microglial cells. Sustained activation of SOCE, possibly mediated by TRP channels, occurred after brief BDNF application and contributed to the maintenance of sustained $[Ca^{2+}]i$ elevation. Pretreatment with BDNF significantly suppressed the release of NO from activated microglia. Additionally, selective serotonin reuptake inhibitors (SSRIs), including paroxetine or sertraline, potentiated the BDNF-induced increase in $[Ca^{2+}]i$ in rodent microglial cells This article provides a review of recent findings on the role of BDNF in the pathophysiology of neuropsychiatric disorders**,** especially by focusing on its effect on intracellular Ca^{2+} signaling in microglial cells.

Keywords: BDNF, microglia, calcium, TRP channels, inflammation, depression, schizophrenia.

INTRODUCTION

Microglia are the intrinsic immune cells which release many factors, including proinflammatory cytokines, nitric oxide (NO) and neurotrophic factors, when they are activated in response to brain injury or immunological stimuli [1- 4]. Recent *in vivo* imaging has shown that resting microglial cells are highly active with motile protrusions [5] and monitor the functional status of synapses after transient cerebral ischemia [6]. There is increasing evidence suggesting that pathophysiology of neuropsychiatric disorders, such as schizophrenia [7] or depression [8, 9], is related to the inflammatory responses mediated by microglial cells.

Brain-derived neurotrophic factor (BDNF), one of the neurotrophins, has various important roles in cell survival, neurite outgrowth, neuronal differentiation and gene expression in the CNS [10]. These trophic effects of BDNF generally occur over a time course of hours to days. BDNF is required for the induction of long-term potentiation (LTP) at glutamatergic synapses, which accompanies the local mRNA synthesis or the upregulation of immediate early gene activity-regulated cytoskeleton-associated protein (Arc), leading to the formation of hippocampal dendritic spines [10, 11]. Thus, as a possible mechanism underlying trophic effects, BDNF rapidly modulates synaptic transmission within minutes after BDNF application [11- 15]. BDNF is known to induce a rapid increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in neurons [14, 16, 17] and in astrocytes [18]. To date, BDNF is also well known for its involvement in the pathophysiology of neuropsychiatric disorders [19- 23]. There are many reports showing that serum BDNF levels are significantly low in patients suffering from major depression and that BDNF levels are elevated following a course of antidepressants treatment [23, 24]. On the other hand, there is also mounting evidence for an important role of hypothalamic-pituitary-adrenal (HPA) axis abnormalities in the pathophysiology of mood disorders [25]. Chronic stress induces hyperactivity of the HPA axis (hypercortisolism), which leads to the reduction of the BDNF expression in the hippocampus [25]. BDNF attenuates the neuronal death induced by glucocorticoids in the hippocampus of rats [26]. Thus, BDNF also has major roles in the hyperactivity of the HPA axis, which is one of the most extensively studied biological markers for major depression [27].

In the rodent brain, microglial cells express BDNF mRNA [28] and secrete BDNF following stimulation with lipopolysaccharide (LPS) [29]. BDNF promotes the proliferation and survival of microglia themselves [28, 30]. In addition, BDNF released from activated microglia induces

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the sprouting of nigrostriatal dopaminergic neurons after the brain injury [31]. Spinal microglial cells stimulated by ATP release BDNF, resulting in a shift in the neuronal anion gradient, which underlies the mechanism of neuropathic pain [32]. BDNF is also released from neurons [10] or astrocytes [18]. Thus, BDNF is a crucial signaling molecule among microglia, astrocytes and neurons.

In the CNS, intracellular Ca^{2+} signaling regulates many different cellular functions, such as cell proliferation, gene transcription or exocytosis at synapses [33]. Elevation of $[Ca^{2+}]i$ is important in activation of microglial cell functions, including proliferation, release of NO and cytokines, migration, ramification and deramification [34, 35]. Recently, Trang *et al*. showed that influx of extracellular Ca^{2+} causes the release of BDNF from microglial cells [36] and it has been shown that alteration of intracellular Ca^{2+} signaling underlies the pathophysiology of neuropsychiatric disorders, including schizophrenia [37, 38], depression and bipolar disorder [39]. In lymphoblastoid cells derived from patients with bipolar disorders, intracellular Ca^{2+} responses to thapsigargin, an inhibitor of Ca^{2+}-ATPase, are shown to be enhanced [40]. Warsh *et al.* found that chronic treatment with lithium [41] or valproate [42] significantly attenuates lysophosphatidic acid (LPA)-stimulated and thapsigargin-induced intracellular Ca^{2+} responses in B lymphoblast cell lines (BLCLs) from bipolar I disorder (BD-I) patients, suggesting that modulation of intracellular Ca^{2+} mobilization is important for the therapeutic action of mood stabilizers. We have recently reported that pretreatment with antidepressants [43. 44] or antipsychotics [45, 46] significantly inhibited the release of NO and cytokines from activated microglia through the modulation of intracellular Ca^{2+} mobilization. These reports indicated that agents that are able to inhibit microglial activation could be useful for the treatment of neuropsychiatric disorders. This article provides a review of recent findings on the role of BDNF in the pathophysiology of neuropsychiatric disorders**,** especially focusing on its effect on intracellular Ca^{2+} signaling in microglial cells.

BDNF INDUCES SUSTAINED ELEVATION OF INTRACELLULAR CA^{2+} IN RODENT MICROGLIA

BDNF is known to induce a rapid increase in intracellular Ca^{2+} in neurons [14, 16, 17] and in astrocytes [18]. However, there had been no prior reports on how BDNF affects intracellular Ca^{2+} mobilization in microglial cells. We have recently shown that BDNF induces a sustained increase in $[Ca^{2+}]i$ through binding with the truncated Trk B receptor, resulting in activation of the phospholipase C (PLC) pathway and store-operated calcium entry (SOCE) in rodent microglial cells. RT-PCR and immunocytochemical techniques revealed that truncated TrkB-T1 receptors were highly expressed in rodent microglial cells. Sustained activation of SOCE, possibly mediated by TRP channels, occurred after brief BDNF application and contributed to the maintenance of sustained $[Ca^{2+}]i$ elevation. Pretreatment with BDNF significantly suppressed the release of NO from activated microglia [47]. We also observed that pretreatment

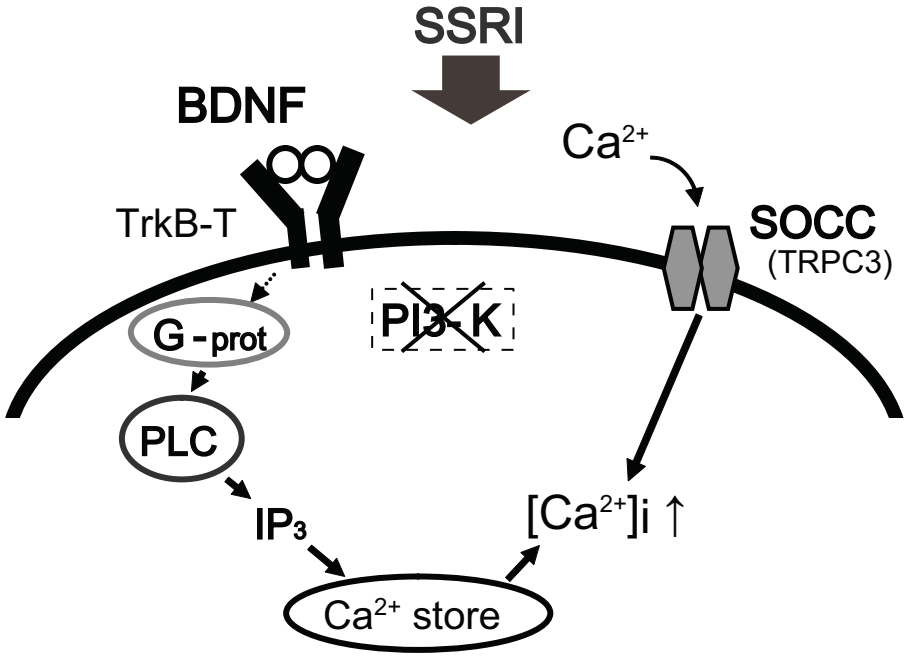

Fig. (1). Schematic illustration demonstrating the underlying mechanism of BDNF-induced sustained increase of $[Ca^{2+}]i$ in rodent microglial cells. BDNF induces a sustained increase in $[Ca^{2+}]i$ through binding of the truncated TrkB receptors (TrkB-T), resulting in activation of the PLC pathway and SOCE. Sustained activation of SOCC, such as TRPC3 channels, occurs after a brief treatment with BDNF and contributes to the maintenance of BDNF-induced sustained intracellular Ca^{2+} elevation. In addition, pretreatment with antidepressants including SSRIs could potentiate the BDNF-induced sustained increase of $[Ca^{2+}]i$ in rodent microglial cells. PLC, phospholipase C; G-prot, G protein; SOCC, store-operated calcium channel; PI3K, phosphatidylinositol 3 kinase; TRPC3, transient receptor potential canonical 3.

with selective serotonin reuptake inhibitors (SSRIs), including paroxetine or sertraline, potentiated the BDNF-induced increase in $[Ca^{2+}]i$ in rodent microglial cells (Mizoguchi, unpublished observations) (Fig. (**1**)). The concentration of BDNF we used (20 ng/mL) is sufficient to promote the proliferation of microglial cells [28] or to rapidly elevate $[Ca^{2+}]i$ in astrocytes [18] and also in neurons [13, 16].

A brief application of ATP in rodent microglial cells induces only a transient intracellular Ca^{2+} elevation. A low concentration of ATP mainly activates purinergic (P2Y) receptors which lead to the activation of PLC and SOCE, resulting in a transient elevation of $[Ca^{2+}]i$ [48]. BDNF induces a transient intracellular Ca^{2+} elevation in cultured rat astrocytes, through the binding to the truncated TrkB receptors, leading to the activation of PLC and SOCE [18]. We also observed that BDNF activated PLC and SOCE through the binding to truncated TrkB receptors in rodent microglial cells, while BDNF induced a sustained but not transient elevation of $[Ca^{2+}]i$ [47]. Activation of microglia, including proliferation, release of cytokines and reactive oxygen species (ROS), migration, ramification and de-ramification, are frequently accompanied by a sustained increase in $[Ca^{2+}]i$ [34, 35]. Thus, BDNF-induced sustained increases in $[Ca^{2+}]i$ may play an important role in the activation of microglia. In PC12 cells, a 1-2 min treatment of nerve growth factor (NGF) rapidly phosphorylates PLC-γ through the activation of the TrkA receptor tyrosine kinase, in which the autophosphorylation of the TrkA receptor and the phosphorylation of PLC-γ are sustained for up to 30 min and 2 h, respectively [49]. A sustained activation of SOCE, possibly mediated by TRP channels, could occur after a brief treatment with BDNF and then contribute to the maintenance of BDNF-induced sustained intracellular Ca^{2+} elevation in rodent microglial cells [47].

BDNF binds to TrkB, a neurotrophin receptor, and there are three TrkB receptor isoforms in the mammalian brain. The full-length form of TrkB (TrkB-FL) receptor contains a catalytic domain of tyrosine kinase which activates intracellular signaling [50]. Two truncated forms of TrkB (TrkB-T1 and TrkB-T2) receptors possess the same extracellular domain, transmembrane domain and the first 12 intracellular amino acid sequences as the TrkB-FL receptor, while they lack tyrosine kinase activity [51]. In cultured rat astrocytes, the expression of truncated TrkB-T1 receptors is more than 100-fold higher than that of TrkB-FL or TrkB-T2 receptors [18, 52], whilst the expression of TrkB-FL receptors is up-regulated after brain injury [53]. Although the physiological significance of truncated TrkB receptors is still unknown [51, 54], several studies support the importance of TrkB-T1 receptors in the CNS. In cultured rat astrocytes, TrkB-T1 receptors mediate the BDNF-induced elevation of $[Ca^{2+}]i$ [18] and are involved in their morphological changes [52]. Carim-Todd *et al.* have recently reported that TrkB-T1-deficient mice develop normally but have increased anxiety-like behavior, accompanied by morphological abnormalities in the dendrites of neurons in the basolateral amygdala [54]. Ernst *et al.* found that the expression of TrkB-T1 but not of TrkB-FL is significantly decreased in the frontal cortex of suicide completers who suffered from major depression [55]. These suggest that reduction of TrkB-T1 receptors might affect the intracellular Ca^{2+} signaling in glial cells, which could contribute to the occurrence of anxiety disorders or major depression. We need further studies to elucidate the physiological significance of truncated TrkB receptors in rodent microglial cells.

BDNF binds to the TrkB receptor and induces the activation of intracellular signaling pathways, including PLC-γ, phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) [51]. BDNF rapidly activates the PLC pathway, leading to the generation of inositol trisphosphate (IP_3) and the mobilization of intracellular Ca^{2+} from the endoplasmic reticulum [18, 50, 51]. The depletion of intracellular Ca^{2+} stores signals the store-operated Ca^{2+} channels to open, leading to the influx of Ca^{2+}. In microglial cells, an application of glutamate or high $[K^{+}]$out does not elevate $[Ca^{2+}]i$, suggesting that both NMDA subtype of glutamate receptors and voltage-operated Ca^{2+} channels might not play important roles under normal conditions [56, and Mizoguchi, unpublished observations]. Using rat hippocampal slice cultures, BDNF application to the apical dendrites of CA1 pyramidal neurons induces a slow and sustained non-selective cationic current mediated by SKF96365-sensitive TRPC3 channels [57]. TRPC channels are necessary for BDNF to induce chemoattractive turning of the growth cone [58] or to increase dendritic spine density [57] in neurons. In non-excitable cells, such as microglia, influx of Ca^{2+} through the TRP channels plays an important role in intracellular calcium signaling [34, 56, 59] and in many inflammatory processes, including the activation of microglia [60]. TRP channels also contribute to the alteration of intracellular Ca^{2+} signaling in patients suffering from bipolar disorder [39, 60].

Pretreatment with BDNF suppresses the release of NO from cultured rat microglia stimulated by LPS [61]. We observed that pretreatment with BDNF significantly suppressed the release of NO from murine microglial cells activated by IFN-γ [47]. In murine microglial cells, risperidone significantly inhibits the expression of inducible NO synthase (iNOS) [45], thus BDNF might also suppress the expression of iNOS in IFN-γ-stimulated murine microglial cells. The biological effects of INF-γ are elicited through the activation of intracellular signaling pathways, including the JAK-STAT pathway [62]. The phosphorylated STAT1 homodimer translocates to the nucleus and initiates gene transcription [62]. In NIH 3T3 cells, influx of Ca^{2+} induced by INF-γ is required for the ser-727 phosphorylation of STAT1 [63]. We observed that pretreatment with BDNF significantly suppressed the IFN-γ-induced elevation of $[Ca^{2+}]i$, along with a rise in basal levels of $[Ca^{2+}]i$ in rodent microglial cells [47]. A 24 h pretreatment with LPS on cultured murine microglial cells suppressed UTP- or complement factor 5a-induced elevation of $[Ca^{2+}]i$, but increased basal levels of $[Ca^{2+}]i$ [64]. It was also demonstrated that an increase in basal $[Ca^{2+}]i$ is required, but by itself is not sufficient, for the release of NO and cytokines from activated microglia [64]. We have also shown that BAPTA-AM significantly inhibits the release of NO from IFN-γ-activated murine microglial cells [46]. BDNF might

inhibit IFN-γ-induced microglial activation through the suppression of IFN-γ-induced elevation of $[Ca^{2+}]i$ in rodent microglial cells. BDNF-induced elevation of basal levels of $[Ca^{2+}]i$ could regulate the microglial intracellular signal transduction. A recent report by Hall *et al.* demonstrated the implication of the basal level of $[Ca^{2+}]i$ in the activation of rodent microglia, including NO production [65].

CONCLUSIONS AND FUTURE PERSPECTIVES

Microglia are predominantly found in the grey matter, with the highest concentration in the hippocampus, olfactory telencephalon, basal ganglia and substantia nigra of the adult mammalian brain [1]. There is increasing evidence that suggests that pathophysiology of neuropsychiatric disorders, including schizophrenia [7] and depression [8, 9], are related to the inflammatory responses mediated by microglia. BDNF might have an anti-inflammatory effect through the inhibition of microglial activation and could be useful for the treatment of neuropsychiatric disorders [47].

BDNF is most abundantly expressed in the hippocampus and cerebral cortex [23] and is also involved in the pathophysiology of neuropsychiatric disorders [19-22]. The BDNF hypothesis of depression was originally based on findings obtained from rodents indicating that acute or chronic stress decreases the expression of BDNF in the hippocampus and the treatment of diverse classes of antidepressant prevents the action of stress [20]. Because the injection of BDNF into the rodent hippocampus exerts antidepressant-like effects in the forced swim and learned helplessness tests, activation of BDNF signaling in the hippocampus is thought to have antidepressant effects [20]. In cultured rat cortical neurons, pretreatment of antidepressants, imipramine or fluvoxamine, potentiates both BDNF-induced transient increase in $[Ca^{2+}]i$ and BDNF-triggered glutamate release *via* the activation of sigma-1 receptors [66]. We also observed that pretreatment with SSRI, paroxetine or sertraline, significantly potentiates the BDNF-induced elevation of $[Ca^{2+}]i$ in rodent microglial cells

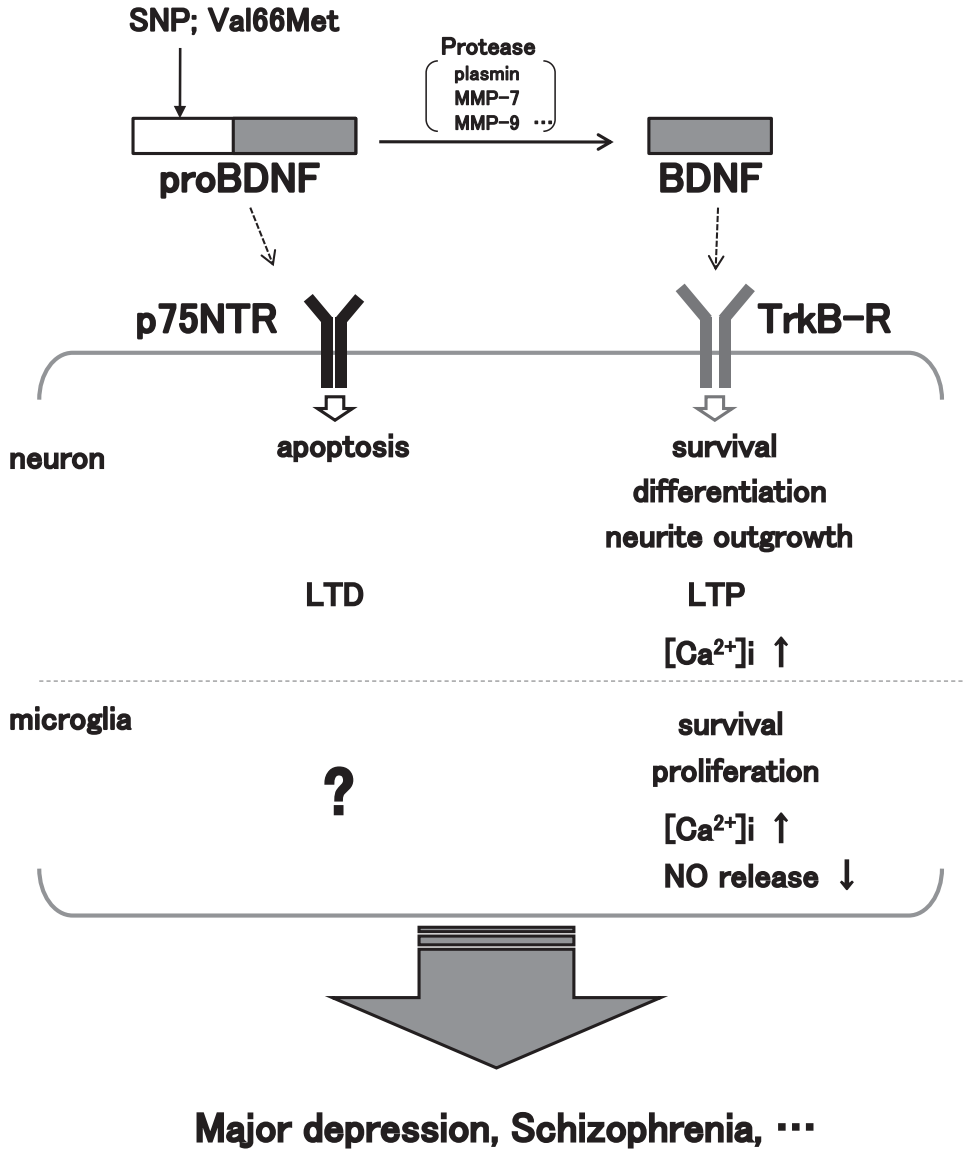

Fig. (2). Schematic illustration showing the opposite effects of proBDNF and BDNF in the CNS. It might be important to examine the effect of proBDNF on microglial cells especially by focusing on the SNP in the pro-domain of the *BDNF* gene.

(Mizoguchi, unpublished observations). Further studies are needed to elucidate the possible involvement of BDNF-induced elevation of $[Ca^{2+}]i$ underlying the mechanism of antidepressant actions. For these purposes, one of key molecules to target might be sigma-1 receptors [67], because sigma-1 receptors are expressed in microglial cells [68]. Interestingly, antidepressants are shown to have diverse (from high to low) affinities for sigma-1 receptors [69].

In the mammalian brain, four neurotrophins including NGF, BDNF, neurotrophin 3 (NT3) and neurotrophin 4 (NT4), have been identified. These closely related molecules act by binding to two distinct classes of transmembrane receptor: the p75 neurotrophin receptor (p75NTR) and the Trk family of receptor tyrosine kinases, which include TrkA, TrkB and TrkC. Like other secreted proteins, neurotrophins arise from precursors, proneurotrophins, which are proteolytically cleaved to produce mature proteins [12]. ProNGF and proBDNF can be cleaved by extracellular proteases, such as plasmin and matrix metalloproteinase 7 (MMP7), to form mature NGF or BDNF [12, 70]. Proneurotrophins bind with high affinity to p75NTR, which for years was considered to be a low-affinity neurotrophin receptor. By contrast, mature neurotrophins bind preferentially to Trk receptors [12]. Interestingly, interaction of mature neurotrophins with Trk receptors leads to cell survival, whereas binding of proNGF or proBDNF to p75NTR leads to apoptosis [12]. In addition, mature BDNF and proBDNF facilitates long-term potentiation (LTP) and long-term depression (LTD) at the hippocampal CA1 synapses, respectively [71]. Thus, Trk and p75NTR preferentially bind mature- and pro-neurotrophins, respectively, to elicit opposing biological responses in the CNS [12, 71]. We observed that p75NTR are expressed in the rodent microglial cells (Mizoguchi, unpublished observations). Minocycline treatment is shown to reduce apoptosis of oligodendrocytes by inhibiting proNGF production by micrglial cells after the spinal cord injury [72]. However, the effects of proBDNF on microglial cells are not fully understood (Fig. (**2**)).

Another important advance came from a study showing that a single-nucleotide polymorphism (SNP) in the pro-domain of the human *BDNF* gene affects the trafficking and secretion of BDNF. This single nucleotide change occurs at nucleotide 196 (G to A), producing an amino acid substitution (valine to methionine) at codon 66 in the pro-domain. Humans with the Met allele have a selective impairment in hippocampus-dependent episodic memory, lower levels of hippocampal *N*-acetyl aspartate (NAA, a putative measure of neuronal integrity and synaptic abundance) and abnormal hippocampal function (as recorded by functional MRI) [73]. Patients suffered from major depression have significantly smaller hippocampal volumes compared with controls [74]. In addition, significantly smaller hippocampal volumes are observed for subjects who carries the Met-BDNF allele compared with those who are homozygous for the Val-BDNF allele [74]. This suggests that Met-BDNF allele carriers might be at risk to develop smaller hippocampal volumes and may be susceptible to major depression [74, 75]. Thus, it will be important to study the effect of proBDNF on microglial cells especially by focusing on the SNP in the pro-domain of the *BDNF* gene (Fig. (**2**)).

7,8-dihydroxyflavone (7,8-DHF) (Fig. (**3**)) has recently been identified as a specific TrkB agonist that crosses the blood-brain barrier after oral or intraperitoneal administration [76]. Systemic application of 7,8-DHF activates TrkB receptors in the rodent amygdala, which results in the enhancement of both the acquisition of fear and its extinction [77]. Whether 7,8-DHF affects microglial cells awaits further investigation.

7,8−Dihydroxyflavone (7,8−DHF)

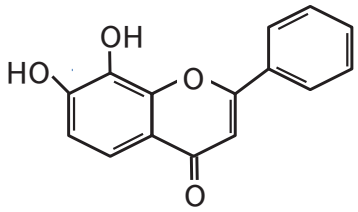

Fig. (3). Chemical structure of the 7,8-dihydroxyflavone (7,8-DHF).

Recently, Raison *et al*. have indicated that disruptions in coevolved relationships with a variety of tolerogenic microorganisms (referred to as old friends) that were previously ubiquitous in soil, food and the gut, but that are largely missing from industrialized societies, might contribute to increasing rates of major depression in the modern world [78]. Loss of exposure to those old friends might promote major depression by increasing background levels of cytokines, including interleukin 1β (IL-1β), tumor necrosis facor α (TNFα) and IL-6, and could predispose vulnerable individuals to inappropriately aggressive inflammatory responses to psychosocial stressors, leading to increased rates of depression [78]. Thus, the cytokine theory of major depression is certainly attractive and the elucidation of the effects of BDNF and/or proBDNF on microglial cells could provide valuable targets for the development of new antidepressant drugs. We have shown direct evidence that rodent microglial cells are able to respond to BDNF, which may be important for the regulation of inflammatory responses, and may also be involved in the pathophysiology and/or the treatment of neuropsychiatric disorders.

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ABBREVIATIONS

BDNF = Brain-derived neurotrophic factor

TrkB = tropomyosin-related kinase B

SOCE = store-operated calcium entry

PLC = phospholipase C

TRP channel = transient receptor potential channel

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