member of the FGF family, and is generally expressed throughout the developing CNS [27-29]. Basic FGF promotes the survival and neurite growth of brain neurons, including norepinephrine and 5-hydroxytryptamine (5-HT), suggesting that it functions as a neurotrophic factor. Furthermore, bFGF has been implicated in both neurogenesis and gliogenesis during development, as well as in adulthood [27-30].

We reported that the serum bFGF levels were significantly higher in schizophrenic patients than in normal controls, and that the serum bFGF levels in patients were significantly correlated with the severity of negative symptoms [31]. Furthermore, we found a significant negative correlation between the serum bFGF levels and the age of onset in the patient group. Our finding of elevated bFGF levels in the serum of patients with schizophrenia, especially in earlier age-of-onset cases considered to have more neurodevelopmental insults, suggests that bFGF abnormalities may be involved in the pathophysiology of schizophrenia [31]. It is suggested that the bFGF produced in astrocytes plays a role in regulating the generation of neurons and astrocytes in the developing CNS [32,33]. Therefore, our findings suggest that the abnormality of bFGF in the regulation of neurons and astrocytes during the developmental stage may be implicated in the pathophysiology of schizophrenia.

Epidermal Growth Factor (EGF)

Epidermal growth factor (EGF) was initially identified as a potent mitogenic peptide with various nonmitogenic activities, including the regulation of cellular migration and differentiation. In the CNS, EGF has been shown to serve as a neurotrophic factor enhancing cell proliferation and neuronal differentiation, and influencing synaptic plasticity, including hippocampal long-term potentiation [34,35]. It has been reported that EGF protein levels are significantly decreased in the postmortem brains of patients with schizophrenia, and that the serum EGF levels in both medicated and drug-free patients with the disorder are also markedly reduced as compared with normal controls [36]. These findings suggest the possibility that impaired EGF signaling might be associated with the pathophysiology of schizophrenia [36]. However, in the study by Futamura *et al.* [36], drug-naive patients (n=4) and chronic patients (n=45; mean age: 47.0 years olds) were included in the analysis of serum EGF levels. Therefore, the results could be accounted for by the effect of antipsychotic medication. We recently found that the serum levels of EGF in drug-naive (n=15) or medicated patients (n=25) with schizophrenia did not differ from those of age- and sex-matched normal controls (n=40) [37]. In contrast, we found a significant correlation ($r = 0.434$, $p = 0.005$) between the serum EGF levels and the Brief Psychiatric Rating Scale (BPRS) score in the combined groups of patients. Therefore, our results do not support the claim that EGF plays a role in the pathogenesis of schizophrenia, but instead suggest that EGF may serve as a state marker, that is, as an index of symptom-linked deficits [37].

The functional polymorphism of the human EGF gene has been demonstrated [38]. Cells from individuals homozygous for the EGF gene polymorphism (61 A allele) produced significantly less EGF than cells from the 61 G homozygotes or heterozygous A/G individuals [38]. Recently, it was reported that the EGF gene polymorphism (G allele) is associated with male schizophrenic patients in Finland, and the G allele was also associated with a later age at onset in male patients with schizophrenia [39], suggesting that the EGF gene may show a susceptibility to schizophrenia. To confirm this, further studies using a large sample with a different population are needed.

Midkine

Midkine is a multifunctional heparin-binding growth factor involved with cell migration, neurogenesis, angiogenesis, and tumorgenesis [40,41]. Recent evidence suggests that midkine plays a role not only in the formation of the nervous system, but also in mental functions [40,41]. Although the mRNA of midkine is strongly expressed in the CNS during the midgestation period, the normal adult brain scarcely expresses it [40,41]. Mice lacking the midkine gene showed disturbed development in the hippocampus and abnormal behavior during infancy [42]. It is therefore of considerable interest to assess the potential contribution of midkine to the pathophysiology of schizophrenia.

We evaluated the serum levels of midkine in drug-naive (n=15) and medicated (n=25) patients with schizophrenia, and age- and sex-matched normal controls (n=38). The patients showed two clusters in their levels of midkine. Four drug-naive patients (26.7%) and two medicated patients (8.0%) had abnormally high values, but no controls did, and there was a significant difference in the numbers [43]. Furthermore, in other patients, the mean levels of midkine in drug-naive schizophrenia were significantly decreased compared to the controls. Thus, there are two clusters of serum midkine abnormalities in drug-naive patients with schizophrenia. The present data, i.e. exclusively high or low serum levels of midkine, suggest that midkine may be implicated in certain maldevelopmental aspects of schizophrenia [43]. In contrast, it has been reported that serum levels of midkine are increased in patients with various types of cancers [44,45], suggesting that serum midkine could serve as a general tumor marker with a good potential for clinical application. In the paper by Shimizu *et al.* [43], patients with tumors were excluded. It should be noted that serum levels in the part of drug-naive patients with schizophrenia are significantly lower than those of normal controls although the specific mechanisms underlying altered midkine levels are currently unclear. Therefore, further studies on serum midkine as a biological marker of schizophrenia should be necessary.

In the brains of human control subjects, some neurons were midkine-immunopositive, but no evident MK-immunoreactivity was observed in the astrocytes. In the ischemic lesions, a significant elevation of midkine-immunoreactivity in the astrocytes and depletion of the reactivity in neurons were seen, especially in the early period [46], suggesting that the midkine in the astrocytes plays some role in the repair process in the early period of ischemic brain lesions in humans. Given the role of midkine as a neurotrophic factor, it is likely that abnormality of the

midkine may play a role in the pathophysiology of schizophrenia.

Brain-Derived Neurotrophic Factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is a 27-kDa polypeptide that is recognized as playing an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and in adulthood. It is also known that BDNF participates in use-dependent plasticity mechanisms such as long-term potentiation and learning [47-52]. Several lines of evidence suggest the role of BDNF in the pathophysiology of psychiatric disorders including mood disorders, schizophrenia, and eating disorders [53-59]. Furthermore, BDNF augmented glutamate-evoked, but not acetylcholine-evoked, currents 3-fold, and increased N-methyl-D-aspartate (NMDA) receptor open probability [60,61]. In addition, the NMDA receptor antagonist MK-801 blocked BDNF enhancement of the synaptic transmission, suggesting that BDNF modulates synaptic efficacy *via* changes in NMDA receptor function [60]. Based on the possible interaction of BDNF with NMDA receptors, it is of interest to examine the role of BDNF in the pathophysiology of schizophrenia.

It has been demonstrated that the levels of BDNF were elevated specifically in the anterior cingulate cortex and hippocampus of schizophrenic patients [62]. In contrast, Durany *et al.* [63] reported that the levels of BDNF were significantly increased in the frontal cortex, parietal cortex, temporal cortex, and occipital cortex of schizophrenic patients, and that the levels of BDNF in the hippocampus were significantly decreased as compared with the controls [63]. Thus, the two studies on BDNF levels in the hippocampus reported opposite results. In addition, it has been reported that the expression of BDNF mRNA and TrkB mRNA in the prefrontal cortex of schizophrenia is significantly decreased as compared with normal controls (Hashimoto *et al.* 32nd Annual Meeting of Society for Neuroscience 2002). More recently, it has been demonstrated that the levels of BDNF mRNA (23 %) and BDNF protein (40 %) in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia are significantly decreased as compared with normal controls, and that neuronal BDNF expression was decreased in layers III, V and VI in the DLPFC of patients, suggesting that intrinsic cortical neurons, afferent neurons, and target neurons may receive less trophic support in schizophrenia [64]. Taken together, these findings using postmortem samples suggest that an alteration in BDNF may be one of the factors which must be considered in the pathophysiology of schizophrenia. However, the levels of BDNF in the brain could be altered by the chronic administration of antipsychotic drugs. Chronic (4 week) administration of the typical antipsychotic drug haloperidol (1 mg/kg) significantly down-regulated BDNF mRNA expression in both the CA1 and dentate gyrus regions of the rat brain compared with the vehicle control. In contrast, chronic (4 week) administration of the atypical antipsychotic drugs clozapine (10 mg/kg) and olanzapine (2.7 mg/kg) significantly up-regulated BDNF mRNA expression in the CA1, CA3, and dentate gyrus regions of the rat hippocampus compared with their respective controls [65]. These findings suggest that typical and atypical antipsychotic drugs differentially regulate the BDNF mRNA expression in the rat hippocampus. Therefore, we cannot rule out the possibility that alterations of the BDNF levels in the brain are induced by antipsychotic drugs rather than the psychosis itself.

High levels of BDNF are detected in human blood, and human platelets have been shown to express BDNF mRNA and contain biological activity ascribed to the BDNF protein [66,67]. Interestingly, a strong correlation was shown between the serum 5-HT levels, a marker for platelet activation, and the serum BDNF levels, suggesting that BDNF may be released by platelets during the clotting process [67]. It has been reported that the serum BDNF levels in chronic patients with schizophrenia are significantly decreased as compared with normal controls [68]. However, no changes in the serum BDNF levels were detected among drug-nave patients, medicated patients, and normal controls [69]. In contrast, the baseline serum BDNF levels of schizophrenic patients were significantly lower than those of controls when patients who failed to meet 30% improvement on their Positive and Negative Syndrome Scale (PANSS) scores were excluded from the study [70]. This result supports the view that lower levels of serum BDNF might be associated with patients who showed improvement on antipsychotic medication [70]. Recently, a positive correlation between the cortical BDNF levels and serum BDNF levels has been reported in rats [71]. In addition, it has been demonstrated that BDNF can cross the blood-brain barrier [72]. Taken together, it is likely that the serum BDNF levels may reflect the BDNF levels in the brain. In addition, the serum BDNF levels may in part participate in the contribution of BDNF to neuronal development and maintenance in the brain. Further studies on the precise mechanisms underlying the role of BDNF in the pathophysiology of schizophrenia are necessary.

The neurodevelopmental hypothesis of schizophrenia makes the BDNF gene an attractive candidate as a susceptibility locus for molecular analysis. The gene of BDNF is assigned to chromosome 11p13 [73], and a dinucleotide repeat polymorphism (166-174 bp) maps 1040 bp upstream of the transcriptional site [74]. Some studies have reported no evidence for the association between the polymorphism of BDNF and schizophrenia [75-78]. A dinucleotide repeat polymorphism has been considered a simple CA (or GT) repeat, but we recently found that this repeat consists of three different and continuous dinucleotide repeats [-$(GC)_{n1}$-$(AC)_{n2}$-$(AG)_{n3}$-] [79]. Therefore, reanalysis of these microsatellite markers is needed to examine their role in the pathogenesis of schizophrenia. Krebs *et al.* [80] reported that an excess of the 172-176 bp alleles was found in patients with late onset, in neuroleptic-responding patients, and in non-substance-abusing patients, suggesting that the BDNF gene variants could influence the phenotypic expression of schizophrenia in relation to predisposition to substance abuse. Recently, the frequency of heterozygous for the C270T allele was approximately twice as high as those observed in the controls, suggesting that the 270T allele may confer susceptibility to schizophrenia [81,82]. However, further studies are needed to support these findings, and, if replicated, to determine whether it is confined to schizophrenia or represents a more generalized developmental process.

Cytokines

Accumulating evidence suggests that, in some cases, schizophrenia is accompanied by changes in the immune system [23-26]. Cytokines have been one of the recent focal points of immunological research in schizophrenia. The CSF levels of interleukin-2 (IL-2), but not interleukin-1α (IL-1α), were found to be higher in 10 neuroleptic-free schizophrenic patients than in 10 healthy subjects matched for sex and age, suggesting that elevated levels of IL-2 in CNS might contribute to the increased dopaminergic neurotransmission, autoimmune phenomena, and abnormal brain morphology described in some patients with schizophrenia [83]. The mitogen-stimulated production of IL-2 was significantly lower in drug naive patients than in controls [84]. There was a significant positive correlation between mitogen-stimulated IL-2 production and age at onset, and a significant negative correlation between mitogen-stimulated IL-2 production and negative symptom scores. In multivariate analyses, the predictive power was stronger for age at onset than for negative symptoms. The correlation of lower mitogen-stimulated production of IL-2 with a younger age at onset suggests that this may be a marker for a subtype of the illness or for severity [84].

Maes *et al.* [85] reported the plasma levels of the soluble IL-2 receptor (sIL-2R), soluble suppressor/cytotoxic antigen (sCD8), IL-1 receptor antagonist (IL-1RA), and Clara cell protein (CC16) in normal controls, nonmedicated schizophrenic patients, and schizophrenic patients treated with risperidone or loxapine. The plasma concentrations of IL-1RA were significantly higher in nonmedicated schizophrenic patients than in normal controls. The plasma CC16 levels were significantly lower in nonmedicated and loxapine-treated schizophrenic patients than in normal controls, whereas the risperidone-treated patients had plasma CC16 levels which were not significantly different from the normal controls. The plasma CC16 levels were significantly and positively related to the age of onset of schizophrenia. Plasma sIL-2R was significantly higher in schizophrenic patients who were treated with risperidone than in normal controls and nonmedicated schizophrenic patients. These results show that schizophrenia is accompanied by an activation of the monocytic arm of cell-mediated immunity (i.e., increased plasma IL-1RA) and lower plasma levels of a natural anti-inflammatory and immunosuppressive agent, i.e. CC16, and that the latter may constitute a trait marker of schizophrenia. The results also showed that chronic treatment with atypical antipsychotic agents, i.e., risperidone, may normalize lower plasma CC16 and increase plasma sIL-2R [85].

A significant difference in the plasma levels of interleukin-1α (IL-1α) and IL-6 was found between schizophrenic patients taking antipsychotic drugs and those not taking them. No significant differences in the concentrations of IL-1β, IL-2 and tumour necrosis factor α (TNF-α) were found [86]. These results suggest that treatment with antipsychotic drugs might alter IL-1α and IL-6 production in schizophrenic patients. In comparison with the control group, schizophrenic patients showed significantly higher serum levels of the soluble IL-2 receptor α (sIL-2R α), IL-6 and IL-1RA [87]. Both the protein and mRNA levels of endogenous IL-1RA were specifically decreased in the prefrontal cortex of schizophrenic patients, whereas the IL-1β levels were not significantly altered in any of the regions (prefrontal and parietal cortex, putamen, and hypothalamus) [88]. The reduction of IL-1RA was not correlated with the dose of antipsychotics given to the patients. In contrast, the serum levels of IL-1RA were increased in schizophrenic patients, especially in drug-free patients [88], which is consistent with a previous report [85]. These findings suggest that chronic schizophrenia down-regulates IL-1RA production in the prefrontal cortex, irrespective of its impact on the periphery. It is likely that the reduction of IL-1RA might reflect an immunopathologic trait of the prefrontal region in schizophrenic patients [88].

Numerous data indicate that cytokines activate astrocytes, and that cytokine-stimulated astrocytes can promote the recovery of CNS function. Cytokine-activated astrocytes produce energy substrates and trophic factors for neurons and oligodendrocytes, act as free radical and excess glutamate scavengers, actively restore the blood-brain barrier, promote neovascularization, restore CNS ionic homeostasis, promote remyelination, and stimulate neurogenesis from neural stem cells [89]. Taken together, it is likely that deficits of the immune system *via* cytokines between astrocytes and neurons might be implicated in the pathophysiology of schizophrenia.

GLUTAMATE HYPOTHESIS

Multiple lines of evidence have suggested that a dysfunction in the glutamatergic neurotransmission might be involved in the pathophysiology of schizophrenia [11-14,16,90-93]. This hypothesis has evolved from clinical findings that phencyclidine (PCP) and its congener ketamine, which blocks the NMDA receptor ion channel (Fig. 1), induce a schizophrenia-like psychosis that includes negative and positive symptoms as well as cognitive dysfunction in normal humans, and that PCP exacerbates symptoms in patients with chronic schizophrenia [92, 94-96]. Furthermore, it has been believed that schizophrenia has a neurodevelopmental component, and that the NMDA receptors are critical in guiding axons to their targets during development [97]. Taken together, these findings suggest that endogenous dysfunction of the NMDA receptor-mediated neurotransmission might contribute to the pathophysiology of schizophrenia. The NMDA receptors play a critical role in a variety of physiological and pathophysiological functions, including excitoxotocity, learning, and memory. Furthermore, the disregulation of NMDA receptor-mediated glutamatergic neurotransmission could cause neurodevelopmental decifits. In addition to glutamate, the NMDA receptors are modulated by glycine, D-serine, kynurenic acid, polyamines, glutathione, and specific divalent cations, including magnesium and zinc (Fig. 1). Taken together, it seems that abnormal levels of these endogenous substances might therefore potentially lead to decreased activation of the NMDA receptors.

Glutamate

Kim *et al.* [98] first reported that the CSF levels of glutamate in schizophrenic patients were significantly decreased as compared with normal controls. However,

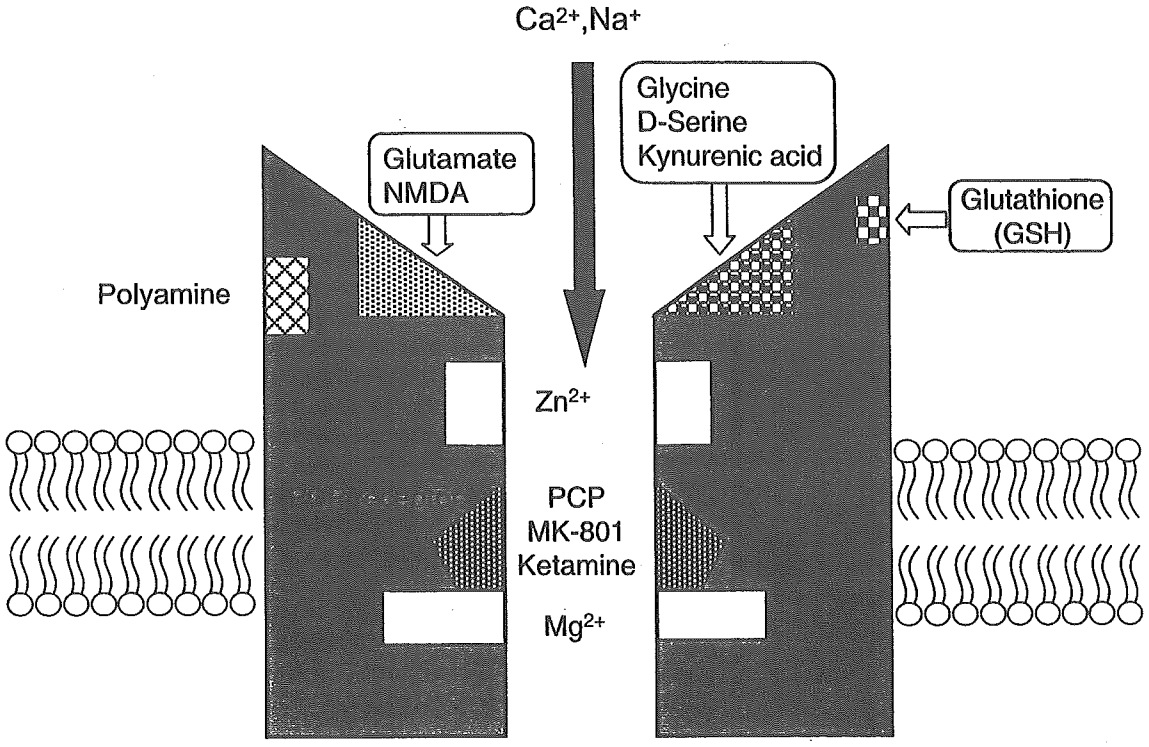

Fig. (1). The NMDA receptor complex. Glutamate and NMDA bind to the agonist site on NMDA receptors. Glycine and D-serine bind to co-agonist site (glycine site) on NMDA receptors, and kynurenic acid binds to co-agonist site as a noncompetitive antagonist. Glutathione (GSH) binds to redox modulatory site on NMDA receptors.

several studies did not find changes in the glutamate levels in the CSF of patients with schizophrenia [99-103, Hashimoto *et al.* 34th Annual Meeting of Society for Neuroscience 2004). Scores of positive symptoms were significantly inversely correlated with the CSF glutamate levels in schizophrenia [103], supporting the potential role of glutamate in the severity of the positive symptoms of schizophrenia. Interestingly, a significant inverse correlation between the CSF glutamate levels and structural and symptomatic features of schizophrenic patients has been reported, suggesting that impaired glutamatergic neurotransmission might be involved in the structural and clinical deficits of schizophrenia [102]. Furthermore, in postmortem studies of schizophrenia, the brain levels of glutamate, aspartate and N-acetylaspartyl glutamate (NAAG) were significantly altered [104]. Notably, the levels of glutamate in the hippocampus and frontal cortex of schizophrenia were significantly decreased as compared with normal controls, whereas the levels of glutamate were found to be increased in neuroleptic-treated controls [104]. Thus, it is unlikely that the CSF levels of glutamate may be sensitive enough to reflect the changes in the defined corticolimbic circuits. It is, therefore, unclear whether the CSF levels of glutamate reflect the actual synaptic activity in the corticolimbic regions.

Glutamate-Glutamine Cycle

In the CNS, glutamine synthesis occurs exclusively in the astrocytes from glutamate and ammonia (Figs. 2 and 3). Glutamine plays major roles in nitrogen and carbon homeostatis, in the detoxification of ammonia, and as a precursor for the synthesis of neurotransmitter glutamate and GABA in specialized excitatory and inhibitory neurons [105-107]. Glutamate is released from presynaptic neurons, and this amino acid can interact with postsynaptic glutamate receptors, including kainate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and NMDA receptors. Released glutamate is taken up by surrounding astrocytes *via* the glutamate transporter (EAAT 1 and 2), where it is converted to glutamine, transported back to the presynaptic neurons, and reconverted to glutamate (Figs. 2 and 3) [105-107].

The development and application of nuclear magnetic resonance spectroscopy (MRS) has permitted the study of carbon and nitrogen metabolism to assess the human brain *in vitro* and *in vivo* using ^{13}C and ^{15}N isotopically labeled substances [105,107]. The 1.5 T MRS study demonstrated that the levels of glutamine were significantly increased in the medial prefrontal cortex of never-treated schizophrenic patients, whereas the levels of N-acetylaspartate (NAA) and glutamate were not altered in these patients [108]. A recent 4.0 T MRS study has shown that the levels of glutamine are significantly increased in the left anterior cingulate cortex and thalamus of never-treated schizophrenic patients, whereas the levels of other substances (NAA, glutamate, taurine, choline, creatine, myo-inositol) were not altered in these patients [109]. These findings of increased glutamine in schizophrenic patients may indicate an abnormality in the cycle of conversion of glutamine to glutamate in the human

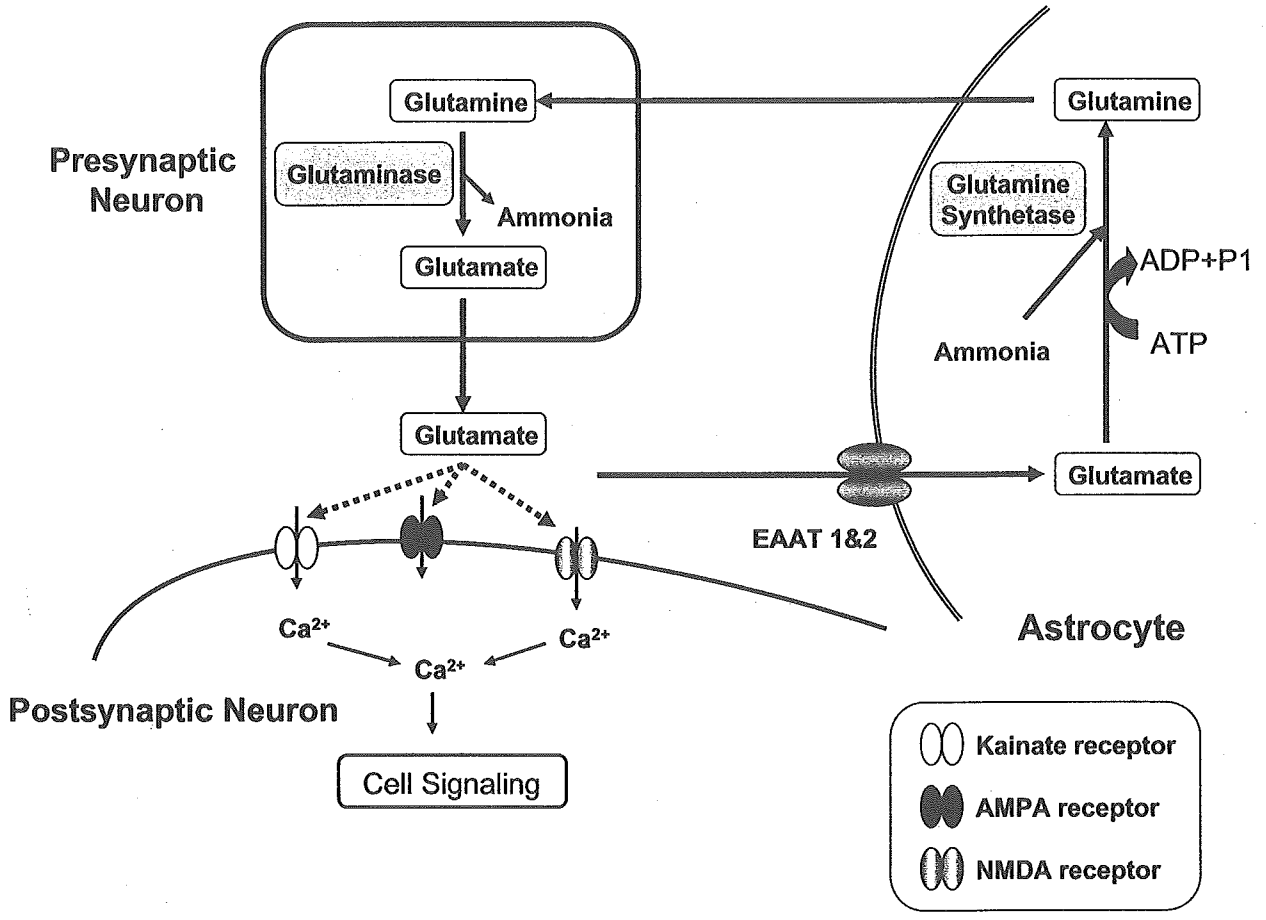

Fig. (2). Glutamate-glutamine cycle in brain. Glutamate released from the neurons binds glutamate receptors (e.g., kainite, AMPA, NMDA) on postsynaptic neurons, and released glutamate is also taken up mostly by the astrocytes *via* the glutamate transporter (EAAT 1 and 2). In the astrocytes, glutamine synthetase converted glutamate to glutamine using ammonia and ATP. The action of glutaminase, which hydrolyses glutamine to glutamate and ammonia within neurons, completes the glutamate-glutamine cycle in the brain.

brain. Recently, we measured the CSF levels of glutamine and glutamate in first episode and drug naive schizophrenic patients and age-matched normal controls. We found that the ratio of glutamine to glutamate in the CSF of the patients was significantly higher than that in the controls, although each level of glutamine and glutamate in the patients was not significantly different from those of the controls (Hashimoto *et al.* 34th Annual Meeting of Society for Neuroscience 2004). As described above, it is unlikely that the CSF levels of glutamate and glutamine may be sensitive enough to reflect the changes in the defined corticolimbic circuits. However, it is possible that the increased ratio of glutamine to glutamate in the CSF sample may reflect impairment of the glutamate-glutamine cycle in the brains of schizophrenia.

In the CNS, the metabolism of glutamate and GABA integrated the synthetic and degradative pathways. Glutamate can be synthesized by glutamate dehydrogenase, GABA transaminase, and glutaminase, a neuronal enzyme most directly involved in the generation of the glutamate pool for neurotransmission (Fig. 3). Glutamate is converted to GABA by glutamic acid decarboxylase, and GABA is metabolized to succinate by the combined reaction of GABA transaminase and succinic semialdehyde dehydrogenase (Fig. 3). The activities of glutaminase and glutamic acid decarboxylase were significantly greater in the DLPFC of schizophrenics than in the comparison group, whereas the activities of glutamate dehydrogenase, glutamine synthetase, and GABA transaminase in the DLPFC of schizophrenia were not different from the controls [110]. These findings suggest that the metabolism of glutamate and GABA might be altered in the DLPFC of schizophrenic patients. In contrast, the activities of glutamine synthetase and glutamate dehydrogenase, the key enzymes involved in the glutamate-glutamine cycle between neurons and astrocytes, were markedly altered in the prefrontal cortex of schizophrenic patients, suggesting abnormalities in the regulation of the glutamate-glutamine cycle in the schizophrenic brain [111]. Given the complexity of the glutamatergic and GABAergic systems in the brain, these findings suggest an abnormality of the glutamate-glutamine-GABA cycle between astrocytes and neurons associated with the NMDA receptor systems in schizophrenia.

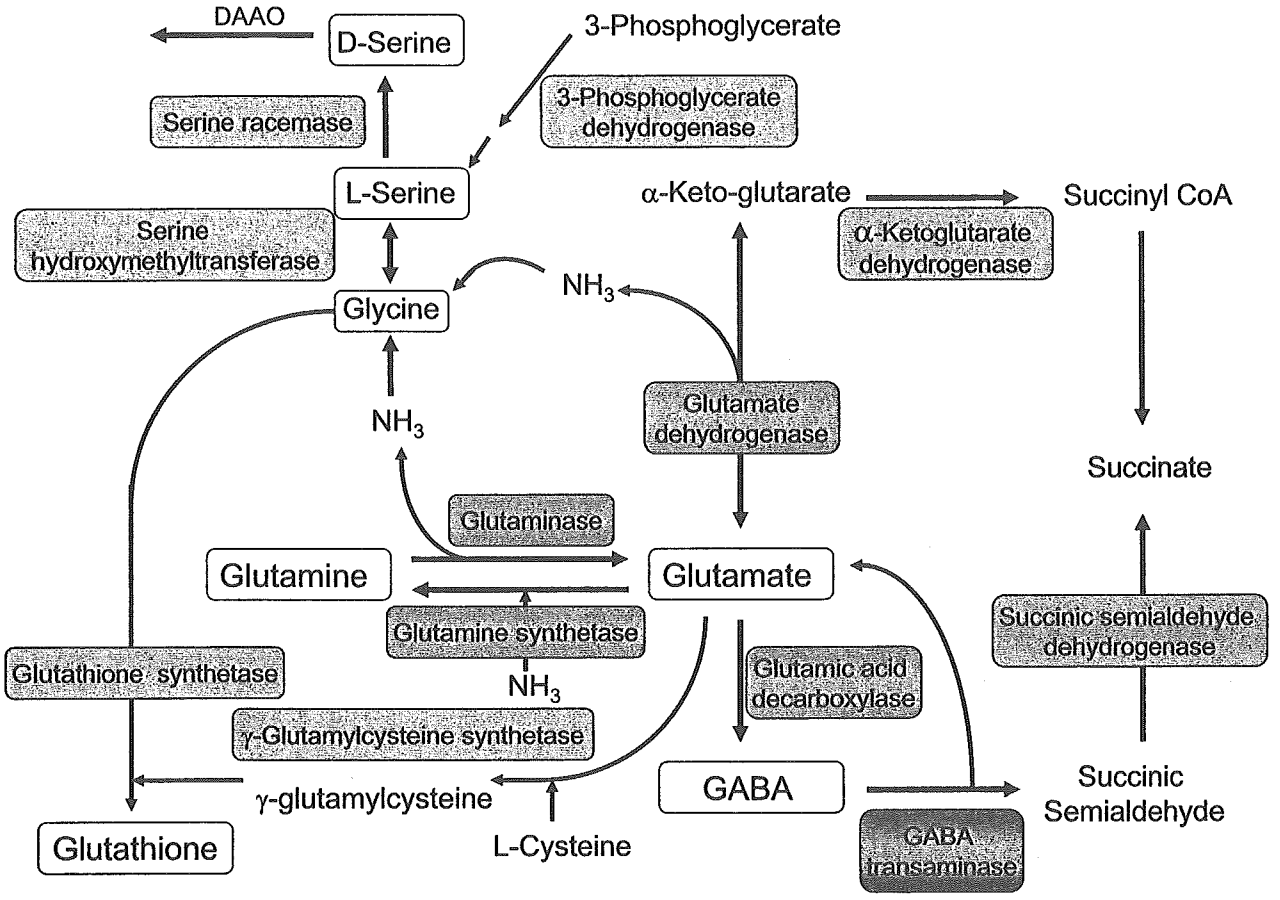

Fig. (3). Enzymatic pathways involved in the synthesis and metabolism of glutamine, glutamate, GABA, glycine, L-serine, D-serine, and glutathione (GSH) in the brain.

D-Serine

The surprising discovery that D-serine, the 'wrong isomer' of serine, occurs naturally in the brain grew out of advances in techniques to assay small quantities of D-serine against the high background of L-serine present in biological samples [112-115]. The high correlation between D-serine levels and NMDA receptors in the rat brain suggested that D-serine might be an endogenous agonist on NMDA receptors [116]. Immunohistochemical studies revealed that endogenous D-serine is localized in the astrocytes of the forebrain grey matter, near or ensheathing the NMDA receptor synapses [117,118]. Agonists of the non-NMDA subtypes of the glutamate receptors promote the release of D-serine from cultured astrocytes [118]. The treatment of brain slices with D-amino acid oxidase (DAAO), which selectively destroys endogenous D-serine but not glycine, reduces NMDA receptor response, suggesting that D-serine occupies glycine sites *in vivo* [119]. The most compelling evidence is the discovery and characterization of the brain enzyme serine racemase, which converts L-serine to D-serine [120,121]. Very recently, it has been demonstrated that knock-out mice for the serine racemase gene show no detectable levels of D-serine in the mouse brain (Coyle *et al.* 34th Annual Meeting of Society for Neuroscience 2004), suggesting the critical role of serine racemase in the production of D-serine in the brain. Taken together, these findings suggest the role of D-serine as an endogenous agonist at the glycine sites on NMDA receptors [122].

Treatment with D-serine revealed significant improvements of positive, negative and cognitive symptoms in patients treated with antipsychotic drugs [123], suggesting the decreased levels of D-serine in the brains of schizophrenic patients. In contrast, treatment with D-serine did not alter symptoms in patients treated with clozapine, although the serum levels of D-serine were significantly increased 6 weeks after D-serine treatment [124], suggesting that the lack of effects of D-serine might be due to the status of antipsychotic drugs. We first reported that the serum levels of L-serine in patients with schizophrenia were significantly increased as compared to normal controls, and that the serum levels of D-serine and the ratio of D-serine to total serine in patients with schizophrenia were markedly decreased in comparison to those of normal controls [125]. In addition, we measured the CSF levels of D- and L-serine in first episode and drug naive schizophrenic patients and age-matched normal controls. We found that the ratio of D-serine to total serine in the CSF of these patients was significantly lower than that of the controls, although each level of D-serine and L-serine in the patients was not significantly different from those of the controls (Hashimoto *et al.* submitted). As described above, it is unlikely that the CSF levels of amino acids may be sensitive enough to

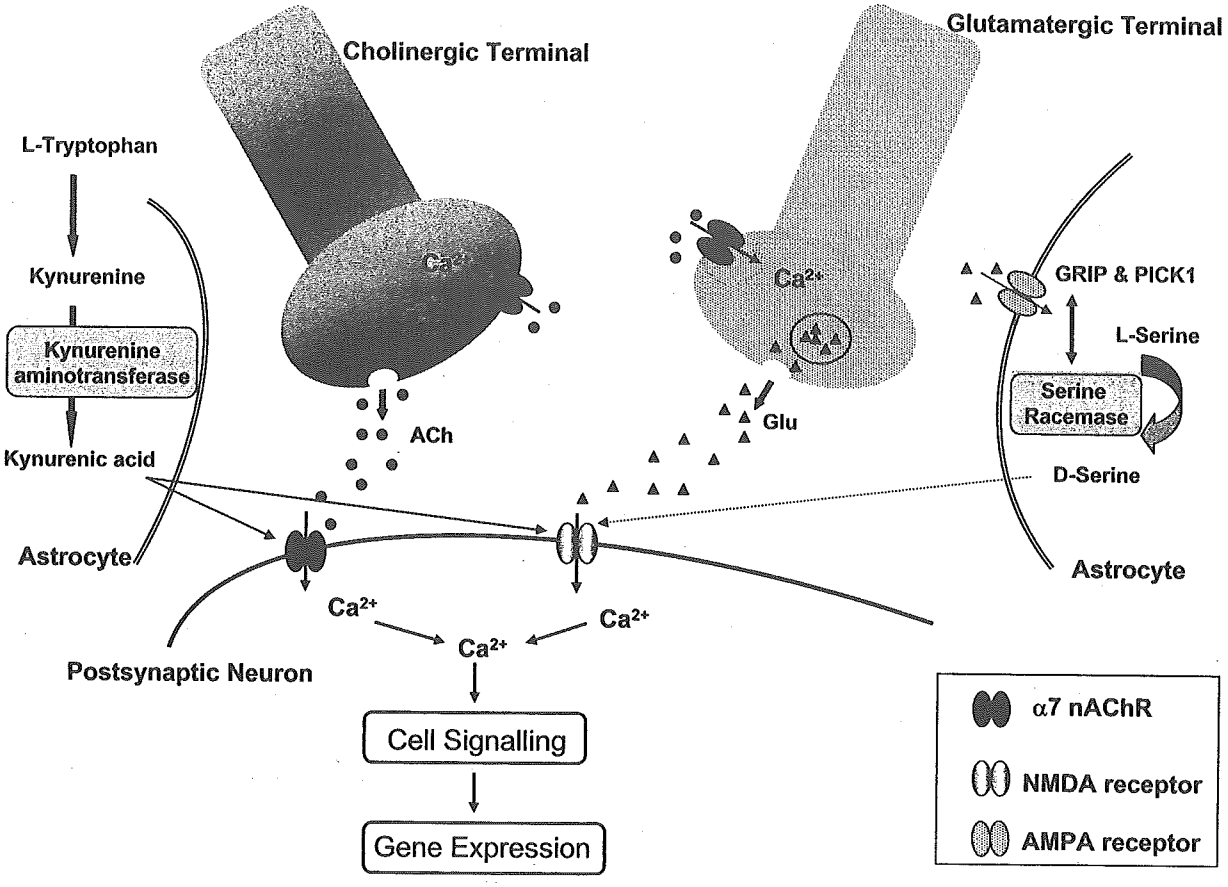

Fig. (4). Role of NMDA receptors and α7 nicotinic receptors (α7 nAChRs) in the neuron-glia communication in the brain. Acetylcholine (Ach) released from the nerve terminal of cholinergic neurons binds to α7 nAChRs on the postsynaptic neurons. By stimulation at α7 nAChRs on the presynaptic terminals, glutamate released from the nerve terminals of glutamate neurons binds to NMDA receptors on the postsynaptic neurons. Thus, the increase in intracellular Ca^{2+} that arises from activation of α7 nAChRs and NMDA receptors lead to cell signaling and gene expression. Kynurenic acid synthesized from L-tryptophan in astrocytes, and, as a non-competitive antagonist, kynurenic acid binds to α7 nAChRs and glycine sites on NMDA receptors. D-Serine is synthesized from L-serine *via* serine racemase in astrocytes. GRIP and PICK1 interact with serine racemase. Glutamate binds to AMPA receptors on astrocyte that stimulate the release of D-serine. Released D-serine binds to glycine site on NMDA receptors. Thus, α7 nAChRs and NMDA receptors can exert a wide range of influences through Ca^{2+} signals, from changes in synaptic plasticity in the brain.

reflect the changes in the defined corticolimbic circuits. However, our CSF data suggests that the synthetic and/or metabolic pathway of D-serine may be impaired in the brains of schizophrenic patients. Measurement of D-serine using the postmortem brains of schizophrenic patients is needed to confirm the role of D-serine in the corticolimbic circuits of schizophrenic subjects. In addition, it has previously been reported that the plasma levels of total serine and glycine in patients with schizophrenia are higher than those of controls [126], and that the levels of serine and glycine in the brains of schizophrenic patients are higher than those of controls [126,127], suggesting a possible abnormality in serine hydroxymethyltransferase, by which glycine is converted to L-serine (Fig. 3). It is also clear that further studies of the relevant synthetic and/or metabolic pathways (e.g., those involving serine racemase, DAAO, serine hydroxymethyltransferase, and 3-phosphoglycerate dehydrogenase) are needed, as are studies of the release and uptake of D-serine, in order to determine the potentially pathological role of decreased D-serine levels in schizophrenia [125].

At the ultrastructual level, D-serine staining appears patchy, and is most abundant around the blood vessels in the forebrain [118]. Furthermore, no changes of D-serine in the forebrain of mice lacking DAAO were demonstrated [128-130], suggesting that DAAO is not thought to regulate D-serine levels in the forebrain where NMDA receptors are abundant. Thus, it is currently unclear how D-serine is metabolized or excreted in the forebrain. It seems that astrocytic D-serine might activate glycine sites of NMDA receptors on the spines of neurons near blood vessels to regulate excitatory neurotransmission. These lines of evidence suggest that D-serine may be swept away by the blood vessels, and the resulting D-serine in the blood may originate from the brain. Therefore, the reduced serum levels of D-serine in schizophrenia may reflect a decrease in the enzymatic activity of serine racemase in the brain of patients [125], although further studies using postmortem brains are needed to confirm the role of D-serine in the corticolimbic circuits.

A recent genetic linkage study has implicated G72 and DAAO in the pathogenesis of schizophrenia, suggesting that changes in the DAAO activity in patients might influence the levels of D-serine, and, by implication, NMDA receptor function [131]. This study proposes a model whereby the expression of G72 in schizophrenia might produce an increase of DAAO activity and a concomitant decrease in the D-serine levels, causing NMDA receptor hypofunction [131]. As described above, DAAO is not thought to regulate the D-serine levels in the forebrain, where NMDA receptors are abundant. Therefore, it is currently unclear how G72 functions in the forebrain, where DAAO is lacking.

Very recently, it has been demonstrated that the glutamate receptor interacting protein (GRIP), which binds AMPA receptors, also binds to serine racemase with the C-terminal amino acids of the receptor binding to the sixth PDZ domain of GRIP [132-134], and that protein interacting with C kinase 1 (PICK1) interacts with serine racemase [135, 136, Hikida *et al.* 34th Annual Meeting of Society for Neuroscience 2004) (Fig. 4). Dr. Snyder's hypothesis was: After glutamate is released from presynaptic terminals, it acts not only on the postsynaptic neurons but also on the surrounding astrocytes. Activation of the AMPA receptors on the astrocytes leads to their phosphorylation by protein kinase C and the dissociation of GRIP. GRIP binds to serine racemase, activating it with marked increases in the formation of D-serine, which leaves the astrocyte to coactivate, with glutamate, NMDA receptors on adjacent postsynaptic neurons [134]. Given the role of D-serine as an endogenous agonist of the NMDA receptors, it is of great interest to study the role of GRIP and PICK1 in the pathogenesis of schizophrenia.

Kynurenic Acid

Kynurenic acid is synthesized *via* kynurenine from the essential amino acid L-tryptophan, and kynurenic acid is produced and released by the astrocytes in the brain [137-141] (Fig. 4). Interestingly, it has been reported that the levels of kynurenic acid are increased in the CSF and postmortem brain of schizophrenic patients [142,143]. In addition to its well-characterized action as a competitive antagonist of the glycine site on the NMDA receptors [144], kynurenic acid also acts as a noncompetitive antagonist of the α7 nicotinic acetylcholine receptors (nAChRs) [145,146], which might be implicated in deficits of auditory sensory gating P50 in schizophrenia [147-151] (Fig. 4). The administration of L-kynurenine, a precursor of kynurenic acid, together with probenecid, an inhibitor of organic acid transport, increased the levels (500-fold) of kynurenic acid in the hippocampus of rats, and also their disrupted auditory sensory gating. In contrast, the administration of L-701,324, a centrally acting antagonist of the glycine site of the NMDA receptors, failed to disrupt auditory gating in rats, suggesting that elevated levels of kynurenic acid produce the disruption in auditory processing through α7 nAChRs [152]. Elevation of the endogenous brain levels of kynurenic acid by the administration of kynurenine (100 mg/kg), the precursor of kynurenic acid, or by the administration of PNU 156561A (10 mg/kg), a potent inhibitor of kynurenine 3-hydroxylase, increased the brain kynurenic acid levels, and significantly reduced prepulse inhibition (PPI) [153]. These disruptions of PPI were restored by administration of the antipsychotic drugs haloperidol or clozapine [153]. These findings suggest that brain kynurenic acid serves as an endogenous modulator of PPI, and are consistent with the hypothesis that kynurenic acid contributes to the pathophysiology of schizophrenia [137,138]. Taken together, it is likely that the blockade of α7 nAChRs in the hippocampus by elevated levels of kynurenic acid might lead to a deficit of auditory sensory gating P50 in schizophrenic patients, and that the disruption of reciprocal astrocyte-neuron signaling mechanisms involving kynurenic acid and α7 nAChRs, and NMDA receptors may play a role in the pathophysiology of schizophrenia (Fig. 4).

Glutathione

Glutathione (GSH; L-glutamyl-L-cysteinyl-glycine), the most abundant thiol present in mammalian cells with concentrations of up to 12 mM, is known as a nucleophilic scavenger and an enzyme-catalyzed antioxidant, and plays an important role in protecting the brain against reactive oxidative stress (ROS) and harmful xenobiotics [154-156]. GSH is synthesized *in vivo* by the consecutive action of two enzymes. γ-Glutamylcysteine synthetase, the rate-limiting enzyme for GSH synthesis, uses L-glutamate and L-cysteine as substrates and forms the dipeptide γ-glutamylcysteine, which is combined with glycine in a reaction catalyzed by the glutathione synthetase to generate GSH (Fig. 3). The balance of cellular synthesis and the consumption of GSH is regulated by feedback inhibition of the γ-glutamylcysteine synthetase reaction by the endproduct GSH [155,156]. Astrocytes appear to contain higher GSH levels than neurons both *in vivo* and in culture. Several studies have convincingly demonstrated that brain astrocytes and neurons strongly influence each other with regards to GSH metabolism and defense against ROS. The importance of astrocytes in the defense of the brain against ROS, and especially the function of GSH metabolism in astrocytes, has become evident, at least for cell culture models. Thus, it is suggested that *in vivo*, a compromised astrocyte glutathione system may contribute to a lower defense capacity of the brain against ROS and subsequently to increased susceptibility to ROS of the astrocytes themselves and of neighboring cells [155,156].

GSH is known to potentiate the NMDA receptor response to glutamate [157] either by acting at redox modulatory site(s) [158] or by blocking high-affinity Zn^{2+} inhibition through Zn^{2+} chelation [159](Fig. 1). Do *et al.* [160] reported that the CSF levels of GSH in drug-free schizophrenic patients were significantly decreased (27 %) compared to the controls. In addition, a non-invasive MRS study demonstrated that the levels of GSH in the medial prefrontal cortex of patients were found to be 52 % lower than in controls [160]. These finding suggest that the impairment of GSH metabolism between neurons and astrocytes may play a role in the pathophysiology of schizophrenia.

GSTM1 of the mu-class glutathione S-transferases (GSTs) catalyze a glutathione conjugate of catechol o-quinones including dopachrome, noradrenochrome, and adrenochrome under physiological conditions. The frequency of the GSTM1 null genotype (decreased levels of GSTMI

activity) was significantly higher among Japanese patients with schizophrenia compared to the controls [161]. Furthermore, the incidence of the GSTM1 null genotype was significantly higher among schizophrenic patients classified as the disorganized type, relative to the control sample. This result was also replicated in a Korean population [162]. These findings suggest that the GSTM1 null genotype is associated with an increased susceptibility to schizophrenia in Asian populations. In contrast, no association between the functional polymorphism (Ile105Val) of the glutathione S-transferase P1 gene (GSTP1) and schizophrenia in the Korean population was reported [163]. The human glutathione peroxidase (GPX1) is a selenium-dependent enzyme which plays an important role in the detoxification of free radicals. The GPX1 gene, which is located on chromosome 3p21.3, may be involved in the pathophysiology of schizophrenia. However, a functional polymorphism, a proline-to-leucine substitution at codon 197 (Pro197Leu) of the human GPX1 gene, was not associated with susceptibility to schizophrenia, suggesting that the GPX1 gene polymorphism is unlikely to be associated with susceptibility to schizophrenia [164].

CONCLUDING REMARKS

An important but unresolved issue is the relationship between neurons and glia in the pathophysiology of schizophrenia [144]. In addition, the molecular mechanisms underlying the pathogenesis of schizophrenia might reflect a meshing of neuron-glia communication and genetic-environmental interactions [165]. As discussed above, multiple lines of evidence suggest that endogenous dysfunction of the NMDA receptor function plays a role in the pathophysiology of schizophrenia. Growth factors, neurotrophic factors, cytokines, amino acids (glutamate, glutamine, L-serine, D-serine), kinurenic acid, and glutathione are diffusible factors between neurons and glia in the brain, whereas a key feature of synaptic plasticity occurs at specific and active synapses. Although several recent findings are both exciting and promising, we are still in the early stages of understanding the role of neuron-glia communication in the pathophysiology of schizophrenia. Further detailed studies should be carried out to elucidate whether abnormality of neuron-glia communication is implicated in the pathophysiology of schizophrenia. Taken together, understanding how endogenous substances (e.g., growth factors, neurotrophic factors, cytokines, glutamate, glutamine, L-serine, D-serine, kynurenic acid, glutathione) could mediate the NMDA receptor function in the neuron-glia communication represents an important direction for future research into schizophrenia. Finally, gaining a further understanding of the role neuron-glia communication plays in the pathophysiology of schizophrenia would provide new perspectives for treating this disease.

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Increased midkine levels in sera from patients with Alzheimer's disease

Ragaa H.M. Salama[a], Hisako Muramatsu[a,b], Eiji Shimizu[c,*], Kenji Hashimoto[c], Shintaro Ohgake[c], Hiroyuki Watanabe[c], Naoya Komatsu[c], Naoe Okamura[c], Kaori Koike[c], Naoyuki Shinoda[c], Shin-ichi Okada[c], Masaomi Iyo[c], Takashi Muramatsu[a]

[a]*Department of Biochemistry and Division of Disease Models, Center for Neural Disease and Cancer, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan*

[b]*Center for Neural Disease and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan*

[c]*Department of Psychiatry (K2), Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuou-ku, Chiba 260-8670, Japan*

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Abstract

Midkine (MK) is a heparin binding growth factor and promotes growth, survival and migration of various cells including neurons. It is also known to accumulate in senile plaques of patients with Alzheimer's disease (AD). To investigate the involvement of serum MK in the pathophysiology of AD, serum MK levels were determined in patients with AD ($n=36$) and age- and sex-matched healthy controls ($n=32$), using an enzyme-linked immunosorbent assay (ELISA). The serum MK values of the patients with AD (median 560 and interquartile range (500–755) pg/ml) were significantly ($U=278.5$, $P=0.0003$, Mann–Whitney U-test) higher than those of the controls (median 500 and interquartile range (385–520) pg/ml). Moreover, 17 patients (47.2%) had abnormally high values of more than 600 pg/ml, but no controls (0%) did. There was no correlation between serum MK level and the mini mental state examination (MMSE) score in the patients. The demonstration of elevated MK levels in sera of patients with AD may contribute toward an understanding the pathophysiology of this disease, and provide a novel potential therapeutic strategy for decreasing neuronal damages in patients with AD. We found that serum MK levels in patients with AD were increased in comparison with those of normal controls.

Keywords: Dementia; Neurotrophic factor; Serum marker

1. Introduction

Midkine (MK), a heparin binding growth factor (Kadomatsu et al., 1988), promotes survival, growth and migration of various cells, including neurons (Muramatsu et al., 1993; Haynes and Rumsby, 2001; Muramatsu, 2002). It is strongly expressed during the midgestation period of embryogenesis, while its expression is nearly undetectable in the normal adult brain (Kadomatsu et al., 1990; Nakamoto et al., 1992; Matsumoto et al., 1994). However, recent evidence suggest that MK may play various roles, not only in the formation of the nervous system, but also in pathological phenomena of the adult brain. Researchers reported that MK expression increases in ischemic brain regions of humans (Yoshida et al., 1995; Wada et al., 2002) as well as rats (Mochizuki et al., 1998). On the other hand, MK has been shown to be neuroprotective (Owada et al., 1999; Harvey et al., 2004). For example, intraventricular administration of MK ameliorates hippocampal delayed neuronal death following transient forebrain ischemia (Yoshida et al., 2001).

Alzheimer's disease (AD) is one of the most prevalent dementing disorders, representing over 50% of all dementia cases in the elderly (Grossberg, 2003). AD is

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HDS-R, Hasegawa Dementia Scale-Revised; MK, midkine; MCI, mild cognitive impairment; MMSE, mini mental state examination; SPECT, single photon emission computed tomography.

* Corresponding author. Tel.: +81 43 226 2148; fax: +81 43 226 2150.
E-mail address: eiji@faculty.chiba-u.jp (E. Shimizu).

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characterized by two pathological hallmarks, namely, senile plaques and neurofibrillary tangles. The former are mainly composed of amyloid-beta peptides (Abeta), while the latter consists mainly of filaments of hyperphosphorylated tau. Yasuhara et al. (1993) reported that senile plaques in the brain of AD were immunoreactive for MK. Furthermore, MK inhibits Abeta fibril formation and Abeta-induced cytotoxicity (Yu et al., 1998; Monji et al., 2000). Since Abeta peptides are believed to play a role in neurodegeneration in AD, MK is likely to play a neuroprotective role in the pathogenesis of AD.

As early detection of AD is beneficial for the treatment of patients, the search for diagnostic markers in serum and cerebrospinal fluid (CSF) has become a rapidly growing field (Teunissen et al., 2002, 2003). Researchers have focused on CSF biochemical markers; total-tau, phospho-tau and Abeta(1–42), to detect AD (Clark et al., 2003; Zetterberg et al., 2003). On the other hand, serum levels of MK are measurable in human samples (Muramatsu et al., 1996), and we have developed highly sensitive enzyme-linked immunosorbent assays to determine MK levels in previous studies (Song et al., 1997; Ikematsu et al., 2000; Shimizu et al., 2003). Because MK is a senile plaque-associated protein, it is of considerable interest to examine serum MK levels in AD cases and healthy controls. Here, the authors report increased MK levels in the sera of AD patients and discuss its significance.

2. Methods

2.1. Subjects

The ethics committee of Chiba University Graduate School of Medicine approved the present study. All of the subjects provided written informed consent for participation in the study after the procedure had been fully explained. Thirty-six patients with AD (mean age: 73.5 years [S.D. 5.8], range: 63–83 years; 13 men and 23 women) were recruited from the Chiba University Hospital and Chiba City Aoba Hospital, Chiba, Japan. Thirty-two age- and gender-matched healthy subjects (mean age: 72.0 years [S.D. 6.5], range: 61–84 years; 8 men and 24 women) also participated in this study as normal controls. All patients were diagnosed as probable AD according to the National Institute of Neurological and Communicational Disorders and Stroke (NINCDS)–Alzheimer's Disease and Related Disorders Association (ADRDA) criteria (McKhann et al., 1984), and underwent a mini mental state examination (MMSE) (Folstein et al., 1975) and/or the Hasegawa Dementia Scale-Revised (HDS-R) (Hosokawa et al., 1994). Patients with cerebral infarcts, hemorrhage, normal pressure hydrocephalus or neoplasm were excluded by brain CT scan or MRI imaging. Single photon emission computed tomography (SPECT) with *n*-isopropyl-*p*-[^{123}I]iodoamphetamine (IMP) or [^{99m}Tc]-labelled L,L-ethyl cysteinate dimer (ECD) was performed as a supplementary means to evaluate the cause of dementia (Johnson et al., 1998). Laboratory tests eliminated the possibility that factors known to change serum MK levels, such as carcinoma, infection, dehydration and hemolysis, were active in the present participants. Patients with any other previous mental or physical illnesses were also excluded from the study. The healthy controls had no history of psychiatric or neurological disorder, and no abnormalities were observed upon routine clinical examination, including a MMSE.

2.2. Procedure

Serum samples of the patients and the controls were collected from 11:00–12:00 am, and stored at −80 °C until assay. The concentration of serum MK was determined by using an enzyme-linked immunosorbent assay (ELISA) as described previously (Muramatsu et al., 1996; Ikematsu et al., 2000). Serum homocysteine content was also determined by homocysteine microplate STE assay Kit (Diazyme laboratories, San Diego, CA) according to the manufacturer's procedure.

2.3. Data analyses

Normally distributed data are presented as mean ± standard deviation (S.D.); data that were not normally distributed are reported as medians with interquartile ranges. Calculations were performed using the statistical software package Statview (Abacus Concepts, Berkeley, CA) and SPSS for windows (SPSS, Chicago, IN). The Fisher's exact test was used for categorical variables, and the Student's *t*-test was employed for the continuous variables. As the serum MK values were not found to have a normal distribution, the difference between the two groups was examined using the non-parametric Mann–Whitney *U*-test. Spearman correlation coefficients were used to examine the association of the serum MK value with the MMSE score or age. A *P* value of less than 0.05 was considered to be statistically significant.

3. Results

There was no significant difference between the AD patients and the healthy controls (Table 1). The serum MK values of the patients with AD (median 560 and interquartile range (500–755) pg/ml) were significantly (U=278.5, P=0.0003, Mann–Whitney *U*-test) higher than those of age-matched controls (median 500 and interquartile range (385–520) pg/ml) (Table 1, Fig. 1). Based on our previous study (Ikematsu et al., 2000), in which none of 135 healthy control subjects showed MK serum values reaching 600 pg/ml, a subject with an MK level higher than 600 pg/ml was defined as having an abnormally high MK. Seventeen out of 36 (47.2%) patients exhibited abnormally high values of

Table 1
Characteristics and serum midkine levels of the patients with Alzheimer's disease and the healthy controls

	Alzheimer's disease	Controls	*P* value
Sex: M/F	13/23	8/24	0.43[a]
Age, years	73.5±5.8	72.0±6.5	0.31[b]
[range]	[63–83]	[61–84]	
MMSE score	17.7±5.5		
[range]	[6–29]		
MK, pg/ml	560 (500–755)	500 (385–520)	0.0003[c]
[range]	(320–6000)	(220–580)	
High-MK [%]	17 [47.2%]	0 [0%]	<0.0001[a]

Age and Mini-Mental State Examination (MMSE) score were shown in the mean±standard deviation (S.D.).
Serum midkine (MK) concentrations (pg/ml) were reported as medians with interquartile ranges.
A subject with MK level higher than 600 pg/ml was defined as a high-MK.
[a] The comparison among two groups was performed using Fisher's exact test.
[b] The comparison among two groups was performed using unpaired *t*-test (two-tailed).
[c] The comparison between two groups was performed using Mann–Whitney *U*-test.

more than 600 pg/ml, but no controls did, there being a significant difference in the numbers (chi-square=20.1, $df=1$, $P<0.0001$, Fisher's exact test) (Table 1, Fig. 1). However, no correlations were demonstrated between the serum MK level and the MMSE score (Spearman $r=0.092$, $n=36$, $P=0.59$) (Fig. 2, Table 2) or age (Spearman $r=-0.137$, $n=36$, $P=0.43$) in the patients (data not shown).

In our study, 5 of 36 patients had MMSE scores, within the normal range more than 24/30 (Table 2). They were diagnosed as incipient AD using neuropsychological tests

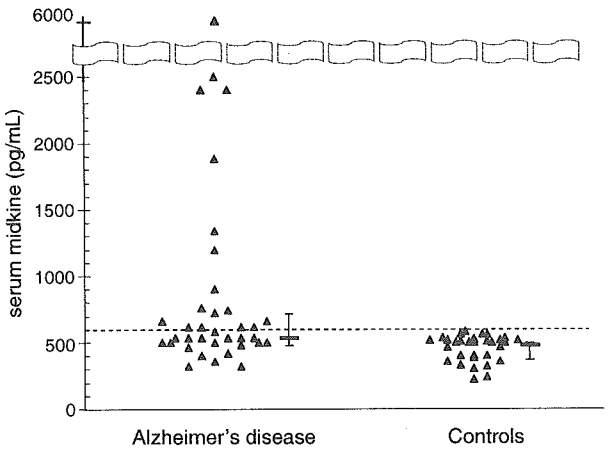

Fig. 1. Distribution of serum midkine (MK) levels (pg/ml) in the patients with Alzheimer's disease (AD) and in the healthy controls. The horizontal bold bars indicate median values of MK, and both side bars show interquartile ranges. The serum MK values of the patients with AD (median 560 and interquartile range (500–755) pg/ml) were significantly ($U=278.5$, $P=0.0003$, Mann–Whitney *U*-test) higher than those of the age-matched controls (median 500 and interquartile range (385–520) pg/ml). The dotted horizontal line indicates the optimal cutoff point (600 pg/ml). Seventeen patients with AD (47.2%) had abnormally high values more than 600 pg/ml, while no controls (0%) did ($P<0.0001$, Fisher's exact test).

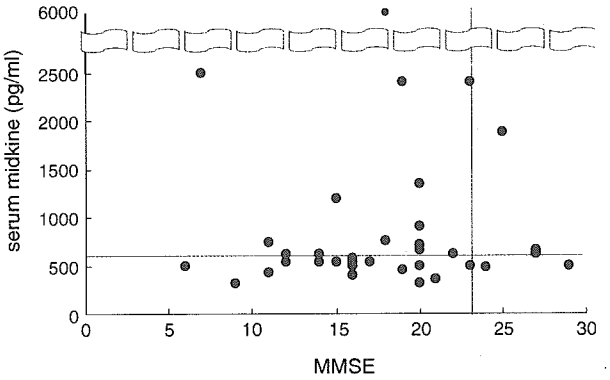

Fig. 2. No correlation between serum midkine (MK) levels and the Mini-Mental State Examination (MMSE) scores in AD patients (Spearman $r=0.092$, $n=36$, $P=0.59$). The horizontal line indicates the optimal cutoff point (600 pg/ml) of MK. The vertical line indicates the optimal cutoff point (23/30) of MMSE. Three of 5 (60%) incipient AD patients with normal MMSE 24/30 or more showed the abnormally high MK value more than 600 pg/ml.

and neuroimaging studies (Table 2). Through longitudinal evaluations, declines of their MMSE scores were confirmed. Interestingly, 3 of the 5 (60%) incipient AD patients with normal MMSE showed abnormally high MK values of more than 600 pg/ml (Table 2). No significant correlation was found between the MK and the homocysteine values (Fig. 3).

4. Discussion

We found that the serum MK levels of the patients with AD were significantly higher than those of the healthy controls. Although MK is scarcely detectable in adult brain (Kadomatsu et al., 1990; Nakamoto et al., 1992; Matsumoto et al., 1994), it is accumulated in senile plaques of the brains of patients with AD (Yasuhara et al., 1993). Since MK binds strongly to Abeta peptides and neutralizes the cytotoxic activity (Yu et al., 1998; Monji et al., 2000), it is possible that MK is induced to counteract the Abeta peptides. These findings of elevated MK levels in the sera and brains of patients with AD suggest that MK may be expressed to prevent Abeta peptide-induced cell death in AD. However, the specific mechanisms underlying the altered serum MK levels in AD patients are currently unclear, and the source of circulating MK remains unknown. In addition to the brain tissues, vascular endothelial cells are considered as candidate sources. Fujisawa et al. (1998) reported that MK was released in time- and heparin-dose dependent manner from cultured vessels, but not from peripheral leukocytes. One possible explanation of the increased serum MK levels observed here would be the increased release of MK from endothelial cells in patients with AD, reflecting a potential abnormality in neurotransmitter release. Another possible explanation would be the parallel MK changes occurring in the brain and serum of AD patients. On the other hand, a breakdown of blood–brain barrier (BBB) and inflammatory

Table 2
Characteristics and serum midkine levels of the patients with Alzheimer's disease

	Sex	Age	MK (pg/ml)	MMSE	HDS-R	Hypoperfusions
A1	M	80	500	6	n.a.	te, pa
A2	F	67	2500 H	7	n.a.	te, pa, pcc
A3	M	81	320	9	n.a.	te, pa
A4	F	72	420	11	n.a.	pa
A5	M	73	740 H	11	6	te, pa
A6	F	74	540	12	8	pcc
A7	F	79	620 H	12	15	pcc
A8	F	69	540	14	13	pcc
A9	F	83	540	14	n.a.	pa, pcc
A10	F	80	620 H	14	10	te, pa, pcc, oc
A11	F	75	540	15	9	te, pa
A12	M	73	1200 H	15	14	te, pa. pcc
A13	F	76	500	16	17	te, pa
A14	F	68	540	16	n.a.	te, pa, pcc
A15	F	82	580	16	n.a.	n.a.
A16	M	65	400	16	12	te, pa, pcc
A17	M	73	540	17	10	te, pa, pcc
A18	F	63	760 H	18	n.a.	te, pa, pcc
A19	M	76	6000 H	18	10	te, pa, pcc
A20	F	81	2400 H	19	17	te, pa, pcc
A21	M	82	460	19	18	te, pa, pcc
A22	F	75	320	20	19	te, pa, pcc
A23	F	64	720 H	20	18	te, pa, pcc
A24	F	70	900 H	20	22	te, oc, pcc
A25	M	65	500	20	16	te, pa, acc
A26	M	65	660 H	20	19	te, pa
A27	M	75	1340 H	20	n.a.	te, pa
A28	F	78	360	21	19	te, pa, pcc
A29	F	67	620 H	22	20	te, pa, pcc
A30	F	69	2400 H	23	19	pa, pcc
A31	M	74	500	23	n.a.	pa
A32	F	75	480	24	24	te, pa, fr
A33	F	80	1880 H	25	20	te, pa
A34	F	74	620 H	27	25	pa, pcc, oc
A35	M	77	660 H	27	17	te, pa, pcc
A36	F	67	500	29	27	te, pa

M, male; F, female. MK, midkine. H, high-MK (higher than 600 pg/ml); MMSE, Mini-Mental State Examination. HDS-R, Hasegawa Dementia Scale-Revised. Hypoperfusions in different brain regions detected by single photon emission computed tomography (SPECT). fr, frontal; te temporal; pa, parietal; oc, occipital; acc, anterior cingulate cortex; pcc, posterior cingulate cortex. n.a., not assessed.

processes may be early events in the pathology of AD, even though Abeta play a prominent role in the initiation and progression of cellular dysfunction in AD. There is a possibility that high serum MK levels reflect a breakdown of BBB in AD patients. As the ability of MK to cross an intact blood–brain barrier and the precise relationship between serum MK and brain MK levels remain unknown, further studies will be needed to determine whether serum MK levels may reflect MK levels in the brain.

Approximately half of AD patients (47.2%) showed abnormally high MK values of more than 600 pg/ml. These results suggest that there may be two different groups of AD patients, some with high-MK (MK-positive AD) and some with normal-MK (MK-negative AD). There was no correlation between the serum MK values and MMSE scores in the AD patients. In addition, we found that 3 of 5 (60%) incipient AD patients with normal MMSE showed abnormally high MK values of more than 600 pg/ml. This suggests that MK-positive AD may not mean early nor advanced stages of this illness, and high-MK may not reflect the severity of cognitive impairment. There was no correlation of serum MK with the disease progression in AD. Based on the previous results about neuroprotective effect of MK (Owada et al., 1999; Yoshida et al., 2001; Harvey et al., 2004), we formulate a hypothesis that the neuroprotective mechanisms may induce MK to inhibit Abeta self-aggregation, and MK may suppress the progress of this disease in acute and chronic neurodegeneration through clinical courses of AD. From the viewpoint, MK-positive AD may make gradual and slower progress, while MK-negative AD may be getting worse rapidly. Further studies including the investigation of the role of MK in progression of AD will be needed. Moreover, the role of elevated serum MK levels in the clinical features in AD is unclear at present. To examine whether serum MK levels may be one of the biomarkers distinguishing normal elderly people from subjects with mild cognitive impairment (MCI) who are likely to develop AD, large-scale studies of serum MK in more subjects with MCI will be necessary.

Hyperhomocysteinemia is a strong risk factor for atherosclerotic vascular disease, and elevated serum homocysteine is correlated with vitamin B deficiency. Significantly elevated homocysteine levels were found in patients with Alzheimer's disease as well as in patients with vascular dementia (McCaddon et al., 1998; Clarke et al., 1998). In this study, no significant correlation was found between serum MK and homocysteine values (Fig. 3). This result suggests that AD with hyperhomocysteinemia and MK-positive AD have different pathophysiological pathways.

It is still unknown whether the increased MK level is specific to AD. Yasuhara et al. (1995) reported that staining for MK, seen in senile plaques in AD, was not seen in Pick's disease. However, ischemic brain regions (Yoshida et al., 1995; Wada et al., 2002), extracellular neurofibrillary tangles in brains of patients with the parkinsonism–dementia complex of Guam (Yasuhara et al., 1996), glial cytoplasmic inclusions of multiple system

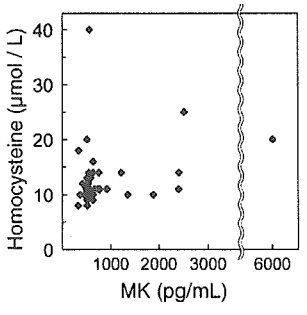

Fig. 3. No correlation between serum midkine (MK) and homocysteine value in AD patients.

atrophy brains (Kato et al., 2000) and human brain tumors (Mishima et al., 1997) also express MK. The Serum MK levels in other irreversible dementias, including vascular dementia, diffuse Lewy body disease, and Pick's disease, should be examined in the future.

5. Conclusions

In this study, we found that serum MK levels in patients with AD were increased in comparison with those of normal controls. Our findings, taken together with previous literature, suggest that MK may be implicated in neuroprotective aspect against the pathophysiology of AD.

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Mini-review

Role of brain-derived neurotrophic factor in eating disorders: Recent findings and its pathophysiological implications

Kenji Hashimoto*, Hiroki Koizumi, Michiko Nakazato, Eiji Shimizu, Masaomi Iyo

Department of Psychiatry, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chiba 260-8670, Japan

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Abstract

Eating disorders, which include anorexia nervosa (AN) and bulimia nervosa (BN), are disorders characterized by abnormal patterns of weight regulation and eating behaviors, and by disturbances in attitudes and perceptions toward weight and body shape. Brain-derived neurotrophic factor (BDNF) plays a critical role in regulating neural survival, development, function, and plasticity in the brain. Recent findings using heterozygous BDNF (±) knock-out (reduced BDNF levels) mice have provided evidence that BDNF plays a role in regulating eating behaviors. Recently, we found that serum levels of BDNF in patients with eating disorders are significantly decreased compared with normal controls. In addition, an association between the BDNF gene polymorphism and eating disorders has been demonstrated. We reviewed the role of BDNF in the pathophysiology of eating disorders and the BDNF gene as a susceptibility gene for eating disorders. Considering the low levels of BDNF in patients with eating disorders, using drugs that increase the BDNF levels and/or BDNF gene therapy are possible novel therapeutic approaches. Providing confirmation that the BDNF gene is the true susceptibility gene for eating disorders could lead to rapid therapeutic progress in treating these disorders. In addition, a more complete understanding of the signal transduction pathway via the p75 neurotrophin receptor ($p75^{NTR}$) and TrkB receptors would provide new perspectives for treating eating disorders.

Keywords: Anorexia nervosa; Brain-derived neurotrophic factor (BDNF); Bulimia nervosa; Eating disorders; Polymorphism

Contents

Abbreviations: AN, anorexia nervosa; BDNF, brain-derived neurotrophic factor; BITE, Bulimic Investigatory Test, Edinburgh; BN, bulimia nervosa; CCK, cholecystokinin; CRH, corticosterone-releasing hormone; HDRS, Hamilton Depression Rating Scale; 5-HT, 5-hydroxytryptamine; NPY, neuropeptide-Y; $p75^{NTR}$, p75 neurotrophin receptor; PPY, peptide-YY; PET, positron emission tomography; tPA, tissue plasminogen activator.

* Corresponding author. Tel.: +81 43 226 2147; fax: +81 43 226 2150.
E-mail address: hashimoto@faculty.chiba-u.jp (K. Hashimoto).

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1. Introduction

People suffering from eating disorders exhibit serious disturbances in eating behavior, such as extreme and unhealthy reduction of their food intake or severe over-eating, as well as feelings of distress about, or extreme concern over, their body shape or weight. Eating disorders frequently develop during adolescence or early adulthood, but some studies have reported their onset during childhood or later in adulthood. Eating disorders affect some 1–3% of women in the United States (Becker et al., 1999; Kaye et al., 2000; Walsh and Devlin, 1998; Fairburn and Harrison, 2003; Klein and Walsh, 2004; Polivy and Herman, 2002). The major types of eating disorders are anorexia nervosa (AN) and bulimia nervosa (BN) that are diagnosed by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Ed. (DSM-IV; American Psychiatric Association; Table 1). The etiology of eating disorders is presumed to be complex, and to be influenced by multiple developmental, social, and biological processes (Becker et al., 1999; Kaye et al., 2000; Fairburn and Harrison, 2003; Walsh and Devlin, 1998; Klein and Walsh, 2004).

Brain-derived neurotrophic factor (BDNF) is recognized as a critical regulator in the survival, differentiation, and outgrowth of select peripheral and central neurons during development and in adulthood, and is also implicated in the synaptic plasticity of such brain functions as learning and memory (Snider, 1994; Thoenen, 2000; Schinder and Poo, 2000; Huang and Reichardt, 2001; Malcangio and Lessmann, 2003; Mattson, 2002; Mattson et al., 2003, 2004; Lu, 2003a). Several lines of evidence suggest that BDNF plays an important role in the pathophysiology of psychiatric diseases, including mood disorders, and in the mechanism of action of therapeutic agents (Duman et al., 1997; Duman, 2002; Tamminga et al., 2002; Manji et al., 2003; Nestler et al., 2002; Coyle and Duman, 2003; Green and Craddock, 2003; Angelucci et al., 2004; Hashimoto et al., 2004).

Table 1
Diagnosis of eating disorders

Diagnostic criteria for anorexia nervosa (AN)

A. Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
B. Intense fear of gaining weight or becoming fat, even though underweight.
C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.
D. In postmenarcheal females, amenorrhea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g., estrogen, administration.)

Specify type

Restricting type: during the current episode of anorexia nervosa, the person has not regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Binge-eating/purging type: during the current episode of anorexia nervosa, the person has regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Diagnostic criteria for bulimia nervosa (BN)

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
   1. Eating, in a discrete period of time (e.g., within any 2-h period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances
   2. A sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating)
B. Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
C. The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.
D. Self-evaluation is unduly influenced by body shape and weight.
E. The disturbance does not occur exclusively during episodes of anorexia nervosa.

Specify type

Purging type: during the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas

Non-purging type: during the current episode of bulimia nervosa, the personas used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas

- Purging Type: during the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas
- Non-purging Type: during the current episode of bulimia nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas

From American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders (Fourth Ed.), American Psychiatric Press.

2. Animal studies

There is also evidence supporting the critical role of BDNF in regulating eating behaviors in animals. For example, heterozygous BDNF (±) knock-out mice (e.g., approximately 50% levels of BDNF of wild-type mice) show enhanced inter-male aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood, and these behavioral abnormalities have been correlated with 5-hydroxytryptamine (5-HT) dysfunction in the brain, since these behavioral abnormalities were ameliorated by chronic administration of the selective 5-HT re-uptake inhibitor fluoxetine (Lyons et al., 1999). Furthermore, heterozygous BDNF (±) knock-out mice exhibit abnormalities in eating behavior or locomotor activity, and infusion of the BDNF protein can transiently reverse abnormal eating behaviors (Lyons et al., 1999; Kernie et al., 2000). To help elucidate the role of BDNF in weight regulation, Rios et al. (2001) have generated conditional mutants in which BDNF has been eliminated from the brain after birth through the