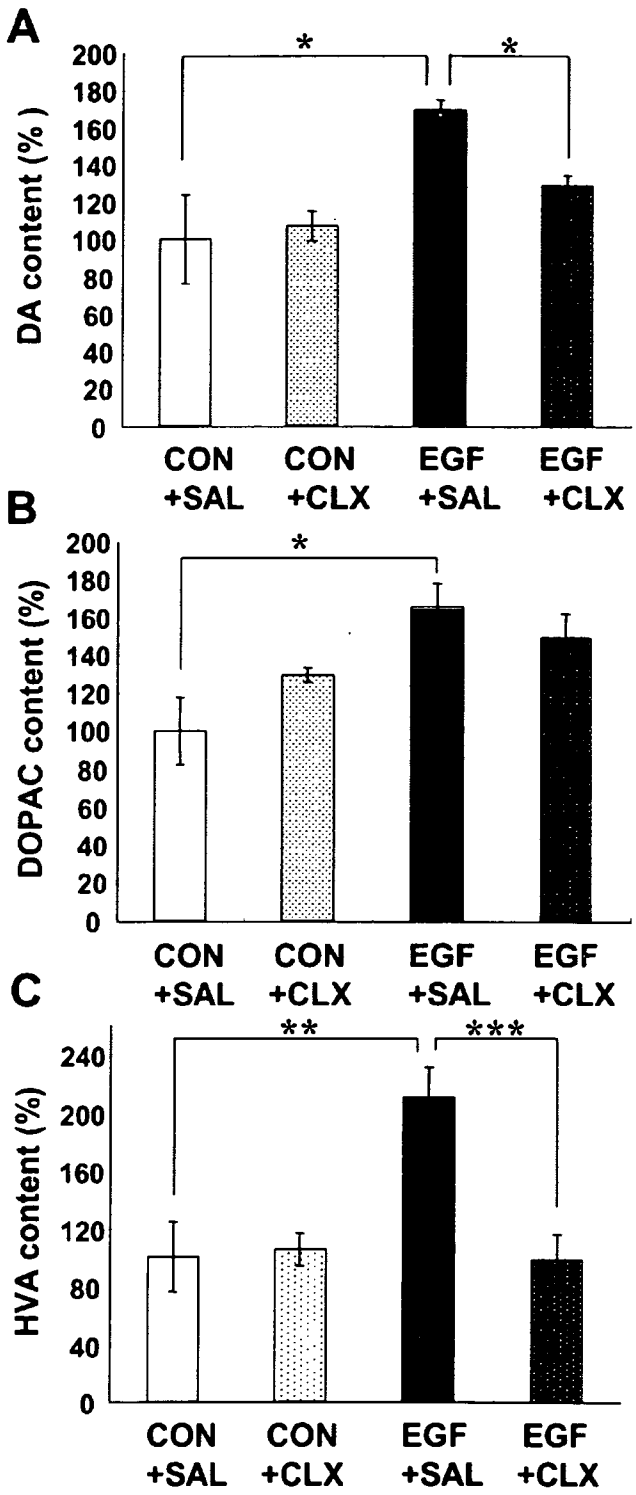

Figure 10. Effects of subchronic celecoxib treatment on dopamine and its metabolites. The levels of dopamine and its metabolites were measured in the striatum of vehicle-infused (CON) and EGF-infused (EGF; 30 μg/pump) control rats that also subchronically received celecoxib (CLX) or saline (SAL). Note that, to minimize acute effects of celecoxib treatment, tissue dissection was performed at least 20 h after the last treatment with celecoxib. White and black bars represent vehicle-infused and EGF-infused rats receiving saline orally. Black dotted and white dotted bars represent vehicle-infused and EGF-infused rats receiving celecoxib orally. Error bars indicate means ± SEM (n = 5–6 each). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Fisher's LSD.

Table 2. Effects of EGF infusion and celecoxib on tyrosine hydroxylase activity in the striatum

	Tyrosine hydroxylase activity		
	CON-SAL	EGF-SAL	EGF-CLX
Dorsal striatum	9.8 ± 0.5	12.1 ± 0.5*	12.3 ± 0.8*
Ventral striatum	9.4 ± 1.0	12.5 ± 0.6*	11.7 ± 0.6*

EGF (30 μg/pump) or saline (CON) was subchronically administered to the striatum of rats. Some rats were orally given celecoxib (CLX) or saline (SAL) 3 d after EGF infusion was initiated. The celecoxib treatment was done daily and repeated for 7 d. Tissue homogenates were prepared from the ipsilateral hemisphere of the striatum, and the activity of tyrosine hydroxylase was measured by monitoring the production of L-DOPA. Data represent means ± SEM (picomoles per milligram of protein, n = 6 animals each). *$p < 0.05$ compared with saline-infused control rats (CON-SAL) by Fisher's LSD

tion were significantly lowered by subchronic treatment with celecoxib.

How does striatal EGF infusion trigger dopamine-associated behavioral deficits and elevate dopamine levels without changing the expression of the rate limiting enzyme for dopamine synthesis, tyrosine hydroxylase? There are reports that inflammatory cytokines including EGF can elevate the activity of tyrosine hydroxylase by phosphorylating the enzyme or upregulating its cofactor tetrahydrobiopterin (Halegoua and Patrick, 1980; Anastasiadis et al., 1997). We measured the tyrosine hydroxylase activity in the dorsal and ventral striatum of the rats receiving EGF in the striatum (Table 2). The activity of tyrosine hydroxylase in the dorsal striatum of EGF-infused animals was significantly larger than that of saline-infused controls ($p = 0.017$). An increase in the enzymatic activity was also detected in the ventral striatum ($p = 0.012$). However, subchronic cotreatment with celecoxib did not attenuate the increase in the activity of tyrosine hydroxylase. We conclude that the increase in dopamine content observed after EGF infusion is, in part, attributable to increased enzymatic activity of tyrosine hydroxylase.

Discussion

The alterations in inflammatory cytokine levels and the effectiveness of anti-inflammatory drugs observed in schizophrenia patients are consistent with the neuroinflammatory hypothesis of schizophrenia (Muller et al., 2000; Nawa and Takei, 2006). Here we combined the evidence that EGF is an inducer for Cox-2 expression (Ackerman et al., 2004; Slice et al., 2005) with our previous finding that EGF signaling might be upregulated in the striatum of schizophrenia patients (Futamura et al., 2002) and hypothesized that enhanced EGF signaling might increase prostaglandin synthesis and lead to behavioral deficits. Therefore, we tested the neurobehavioral consequences of EGF stimulation in rat striatum as well as the antagonistic effects of Cox-2 inhibition. Subchronic infusion of EGF and concomitant celecoxib treatment produced the following results: (1) striatal infusion of EGF yields behavioral deficits in PPI and latent inhibition of fear learning; (2) these deficits were reversible and extinguished by cessation of EGF infusion; (3) EGF administration elevated the expression of Cox-2, the enzyme activity of tyrosine hydroxylase, and dopamine turnover in the striatum; and (4) subchronic treatment with a Cox-2 inhibitor ameliorated these behavioral deficits and concomitantly normalized dopamine turnover. These observations strengthen the argument that EGF-mediated neuroinflammation may, at least in part, result in abnormal dopamine transmission and associated behavioral deficits.

Enhanced EGF signaling in the striatum and behavioral impairments

The present findings might illuminate the neuropathological implication of abnormal levels of EGF or its receptor in the brain of

schizophrenia patients (Futamura et al., 2002). The postmortem study demonstrates that, among various ErbB1 ligands, EGF content is specifically decreased in the striatum of schizophrenia patients. Whether this decrease in EGF content reflects enhanced release of EGF from vesicular stores or decreased EGF synthesis is not clear. To address this question, here, we exogenously supplied EGF to the striatum: subchronic infusion of EGF into the striatum induced behavioral impairments. Gross learning ability of EGF-infused rats was normal in active-avoidance test (Fig. 8) as well as in contextual fear conditioning (Fig. 9), however. Abnormalities in PPI as well as in latent inhibition are often observed in schizophrenia patients (Gray et al., 1995; Weiner and Feldon, 1997; Braff et al., 2001; Swerdlow et al., 2001). Accordingly, these results suggest that elevated EGF/ErbB1 signaling in the striatum might contribute to etiology or pathology of schizophrenia.

EGF and other ErbB1 ligands elevate the expression of tyrosine hydroxylase (Casper et al., 1994; Farkas et al., 2002; Iwakura et al., 2005). EGF also modulates the activity of tyrosine hydroxylase by promoting phosphorylation of the enzyme or increasing the synthesis of its essential cofactor tetrahydrobiopterin (Halegoua and Patrick, 1980; Anastasiadis et al., 1997). There was a significant increase in its enzymatic activity in EGF-infused animals. Because this enzyme limits the rate of dopamine synthesis, this increase in enzymatic activity presumably led to an increase in the synthesis and release of dopamine. In this context, the increase in DAT expression might result from enhanced dopamine release through the negative feedback regulation (Xia et al., 1992; Fang and Ronnekleiv, 1999).

Subcutaneous administration of EGF to neonatal rats and mice increases dopamine turnover and later results in life-long neurobehavioral deficits (Futamura et al., 2003; Tohmi et al., 2005). In that experimental paradigm, however, there is a large time lag between the dopaminergic abnormality in neonates and the emergence of the behavioral impairments in adults. Here we learned that striatal EGF infusion to adult animals similarly perturbed dopaminergic responses and mimicked the behavioral deficits induced by neonatal treatment with EGF. Thus, the behavioral deficits induced by neonatal EGF treatment might share a common pathologic mechanism with those of the present striatal EGF infusion, although the persistency of the deficits differs significantly.

Behavioral deficits associated with elevated dopamine synthesis and metabolism

Consistent with these reports, the present experiments demonstrate that EGF-triggered behavioral abnormalities are concomitant with changes in dopaminergic metabolism in the striatum. EGF administration increases the concentrations of dopamine and its metabolites in the striatum and impaired prepulse inhibition performance in a dose-dependent manner, and, conversely, celecoxib treatment normalized the levels of this neurotransmitter and metabolites, as well as behavioral performance. This elevated dopamine metabolism, but not the increase in the tyrosine hydroxylase activity, was reversed by subchronic treatment with the Cox-2 inhibitor celecoxib. Previous reports demonstrated that prostaglandins influence the activity of excitatory neurons and monoaminergic neurons (Takechi et al., 1996; Oida et al., 1997; Nakamura et al., 2001; Matsuoka et al., 2005; Sang et al., 2005). Given the defined roles of EGF and prostaglandin, we speculate that EGF may increase dopamine synthesis and prostaglandins may enhance its release. These two effects may act synergistically to induce the observed behavioral deficits, although these explanations need to be verified in future experiments including *in vivo* microdialysis.

Targets of EGF in the brain

Lesion studies indicate a major contribution of inhibitory striatopallidal circuitry to the acoustic startle reflexes regulating PPI (Swerdlow et al., 1990, 2001). Unilateral dysfunction of this inhibitory circuitry appears to be sufficient to impair PPI responses (Li et al., 1998; Uehara et al., 2007). In agreement with these reports, both unilateral and bilateral infusion of EGF similarly decreased PPI in the present study.

Enhanced dopamine signaling in both ventral and dorsal striatum is implicated in PPI deficits (Kodsi and Swerdlow, 1995; Swerdlow et al., 2001). Among the striatal regions, the nucleus accumbens is suggested to play an important role in regulating PPI (Swerdlow et al., 1990, 2001). Even when EGF was injected to the center of the striatum, EGF immunoreactivity and an increase in the tyrosine hydroxylase activity were also present in the ventral striatum, including the nucleus accumbens. Accordingly, we had expected that EGF infusion directly into the nucleus accumbens would similarly affect PPI. However, it was not the case. We speculate the reasons. Implanting the cannula directly into the nucleus accumbens might produce surgical injury, potentially counteracting the EGF action or perturbing the local neurotransmission. Alternatively, our preliminary result that dopaminergic terminals in the nucleus accumbens express lower levels of ErbB1 may account for the discrepancy (Zheng et al., 2007). Because latent inhibition of learning involves various brain regions and neural circuits including the striatum, the nucleus accumbens, the limbic system, and the cholinergic system (Weiner and Feldon, 1997; Jeanblanc et al., 2003; Peterschmitt et al., 2005), understanding the present discrepancy may require future experiments of greater complexity.

Neurobehavioral and antipsychotic effects of the Cox-2 inhibitor celecoxib

Significant basal levels of Cox-2 are detectable in several brain regions (Tsubokura et al., 1991; Yamagata et al., 1993; Kaufmann et al., 1996). Inflammatory cytokines induce Cox-2 expression after brain injury, ischemia, and hypoxia (Smith et al., 2000; Ackerman et al., 2004). Increasing Cox-2 expression elevates the levels of all five prostaglandins and activates their downstream receptor signaling (Smith et al., 2000). Conversely, Cox-2 inhibitors attenuate post-ischemic cell death or neurodegeneration associated with Alzheimer's disease (Firuzi and Pratico, 2006). Thus, Cox-2 induction and resultant prostaglandin synthesis are often implicated in neurodegeneration, although the neurotrophic and neurodegenerative actions of prostaglandins remain controversial (Hewett et al., 2000; Strauss and Marini, 2002; Liang et al., 2005). The transgenic mouse model for Alzheimer's disease that overexpresses Cox-2 exhibits age-associated working memory deficits and spatial memory impairment, both of which are sensitive to Cox inhibitors (Sharifzadeh et al., 2005; Melnikova et al., 2006). However, the EGF-induced cognitive deficits in the present study do not appear to involve neurodegeneration because markers for neurons and synapses and brain histochemistry were not altered, the behavioral deficits were reversible, and gross learning scores were normal.

There are several reports demonstrating that the antipsychotic effects and neurocognitive improvement are associated with administration of Cox inhibitors (Ho et al., 2006). Add-on therapy of the Cox-2 inhibitor celecoxib to risperidone improves PANSS of patients with schizophrenia compared with those treated with

risperidone alone (Muller et al., 2004; Riedel et al., 2005). A follow-up study indicates the most pronounced therapeutic effects of Cox-2 inhibitors are cognitive improvements (Riedel et al., 2005). Nonspecific Cox inhibitors such as indomethacin and piroxicam also reverse behavioral and cognitive deficits induced by cocaine, amphetamines, and brain inflammation (Reid et al., 2002; Ross et al., 2002; Matsumoto et al., 2004). The present study may reveal aspects of the mechanism underlying the effectiveness of this medication protocol. Oral administration of celecoxib for 1 week normalized PPI deficits, whereas a single oral dose failed to do so. Thus, subchronic suppression of Cox-2 activity is required to exert significant effects in this animal model (Rivest, 1999; Carothers et al., 2006). In addition, there was no significant effect of celecoxib on basal PPI levels in control animals, suggesting that basal Cox-2 expression, which is normally present in the corticolimbic system (Tsubokura et al., 1991; Yamagata et al., 1993; Kaufmann et al., 1996), do not influence PPI. Although it remains to be determined which types of prostaglandins contribute to the behavioral and cognitive impairments observed after EGF administration, these experiments support the hypotheses that, in addition to its role in neurological diseases, inflammation-triggered prostaglandin synthesis and signaling are potential therapeutic targets for schizophrenia and related psychiatric disorders.

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The anthraquinone derivative Emodin ameliorates neurobehavioral deficits of a rodent model for schizophrenia

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Summary. Abnormality in cytokine signaling is implicated in the neuropathology of schizophrenia. Previously, we established an animal model for schizophrenia by administering epidermal growth factor (EGF) to neonatal rats. Here we investigated effects of the anthraquinone derivatives emodin (3-methyl-1,6,8-trihydroxyanthraquinone) and sennoside (bis-[D-glucopyranosyl-oxy]-tetrahydro-4,4′-dihydroxy-dioxo[bianthracene]-2,2′-dicarboxylic acid) on behaviors of this model and EGF signaling. Subchronic oral administration of emodin (50 mg/kg) suppressed acoustic startle responses and abolished prepulse inhibition (PPI) deficits in this rodent model. ANCOVA revealed that emodin had distinct effects on PPI and startle responses. In contrast, sennoside (50 mg/kg) had no effects. Emodin attenuated weight gain initially during treatment but had no apparent effect on weight gain and locomotor activity thereafter. Application of emodin to neocortical cultures attenuated the phosphorylation of ErbB1 and ErbB2. We conclude that emodin can both attenuate EGF receptor signaling and ameliorate behavioral deficits. Therefore, emodin might be a novel class of a pro-drug for anti-psychotic medication.

Keywords: Antipsychotic; behavior; inflammation; ErbB; EGF; schizophrenia

Introduction

Emodin is an anthraquinone derivative, 3-methyl-1,6,8-trihydroxyanthraquinone, that is extracted and purified from rhubarb. This natural compound has been proposed to possess a variety of pharmacological activities including anti-inflammatory, antiviral, hepatoprotective and antiulcerogenic activities (Wang et al. 2001; Huang et al. 2007). Recent molecular studies indicate that this compound attenuates signal transduction of growth factors and cytokines, inhibiting ErbB2, src-family kinases, IkappaB kinase and MAP kinase (Jayasuriya et al. 1992; Kumar et al. 1998; Zhang et al. 1998, 1999; Wang et al. 2001, 2006, 2007; Li et al. 2005; Kaneshiro et al. 2006). Accordingly, emodin has been reported to exhibit anti-tumor activity against adenocarcinomas, leukemias and lung carcinomas (Lee 2003; Su et al. 2005; Muto et al. 2007). Despite the intensive study of emodin in tumor biology, the effects of this compound on the brain or behavioral traits are largely unknown (Gu et al. 2005; Lu et al. 2007).

The EGF receptors, ErbB1, are enriched in midbrain dopaminergic neurons (Seroogy et al. 1994). Abnormal expression or function of ErbB1 and ErbB2 has been implicated in Parkinson's disease and schizophrenia (Futamura et al. 2003; Mizuno et al. 2004; Iwakura et al. 2005; Tohmi et al. 2005). EGF signaling appears to be perturbed in patients with schizophrenia as ErbB1 levels are increased in the striatum of schizophrenia patients (Futamura et al. 2002). Genetic linkage studies also support the contribution of EGF signaling to the etiology and pathology of schizophrenia (Anttila et al. 2004; Hanninen et al. 2007). To study the mechanisms that contribute to the emergence and symptoms of schizophrenia, we established an animal model for schizophrenia by treating neonatal rats with subchronic doses of EGF. Treated rats later exhibit behavioral deficits in prepulse inhibition, social interaction, and exploratory locomotor activity. Some of these behavioral deficits are sensitive to antipsychotic medication (Futamura et al. 2003; Mizuno et al. 2004; Tohmi et al. 2005). In addition, subchronic exposure of neonates to EGF appears to permanently sensitize ErbB1 signaling (Nawa and Mizuno 2006). Thus, as emodin attenuates EGF receptor signal-

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ing, it may also ameliorate these associated behavioral deficits.

In this study, we investigated the effects of emodin and its derivative on the behavioral deficits associated with this EGF model for schizophrenia. Bearing in mind future therapeutic applications, these anthraquinone agents were given orally and the effects on startle responses and prepulse inhibition were examined at acute and/or subchronic phases of drug administration. In parallel, the side effects of emodin administration on weight gain and locomotion were evaluated. In addition, we examined the activity of emodin on EGF receptor signaling in primary neuronal cultures in order to correlate the molecular activity of emodin with its influence on behavior.

Materials and methods

Subjects

Neonatal Sprague-Dawley rats (postnatal day 2, 10 pups/L) were purchased with dams from SLC Co. Ltd (Shizuoka, Japan). Recombinant human EGF (Higeta Syoyu, Chiba, Japan) was dissolved in saline and subcutaneously administered to half of individual litters daily during postnatal day (PND) 2–10 at the nape of the neck at a dose of 0.875 mg/kg of body weight (Futamura et al. 2003). Control littermates received 0.875 mg/kg of cytochrome c (Sigma Chemical Co., St. Louis, MO, USA), and served as controls for all analyses. After PND20, rats were separated according to gender and raised separately (2–3 rats per cage; 25L × 38W × 18Hcm). All rats were maintained under a 12-h light-dark cycle (7:00 on −19:00 off) with free access to food and water.

Schedule of behavioral testing, drug treatment, and dissection

The cytochrome c- or EGF-treated rats were given emodin (5–50 mg/kg; 96–99% pure; Tokyo Chemical Industry Inc., Tokyo, Japan or 90% pure; Sigma Chemical Co.) or sennoside A (50 mg/kg; >90% pure, Wako Chemical Co., Osaka, Japan) as adults (PND56-62). Emodin or sennoside A was sonicated in a 10% lecithin solution (Wako Chemical Co.) at a final concentration of 5 mg/ml. This emulsion of emodin, sennoside, or vehicle (10% lecithin) was administered to rats once a day for 7 days with the aid of an oral zonde for rats (Natume Seisakusho Co. Ltd., Japan). One day after the last administration, rats were subjected to behavioral tests (see below). Alternatively, rats were given emodin orally (50 mg/kg) once and subjected to behavioral tests 3 h later. The given doses were set below the reported toxic amount of emodin (< 80 mg/kg) according to Jahnke et al. (2004). To minimize interactions between independent tests in Figs. 2, 3, and 5, rats were weighed, tested for locomotor activity and then tested for acoustic startle response. In the other experiments, rats were subjected to only one of the schedule tests. Behavioral tests were performed during the day cycle. In total, 6 experimental groups representing 130 rats were used in the present study. All of the animal experiments described here were performed in accordance with the Animal Use and Care Committee guidelines of Niigata University and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

Rat neocortical culture

Whole cerebral neocortices of embryonic rats (Sprague-Dawley, embryonic day 18–19) were treated with papain solution (10 mg/ml; Sigma Chemicals), mechanically dissociated, and seeded onto poly-D-lysine-coated dishes at a density of 500 cells/mm^2 (Takei et al. 2004). Cortical neurons were maintained in Dulbecco's modified Eagle's medium containing 1 mM glutamine and 10% fetal bovine serum. After 6 days, neuronal cultures were pretreated with emodin (0–300 μM; Tokyo Chemical Industry) or sennoside A (0–300 μM; Wako Chemical Co.) for 2 h and challenged with EGF (5 ng/ml; Higeta Syoyu) for 5 min.

Immunoblot analysis

Polyacrylamide electrophoresis (PAGE) and immunoblotting were performed as described previously (Takei et al. 2004). Cells were harvested, lysed, and sonicated in sample buffer (10 mM Tris–HCl, 150 mM NaCl, 2% SDS, 20 mM NaF, 1 mM Na_3VO_4). After centrifugation, supernatant was collected and the protein concentrations were determined. Equal amounts of protein were subjected to SDS-PAGE and transferred to PVDF membranes. Membranes were probed with anti-phosphorylated-ErbB1 (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-phosphorylated-ErbB2 (1:1000, Upstate, Lake Placid, NY, USA), anti-beta-actin antibodies (1:10000, Chemicon Int, Temecula, CA, USA), followed by horseradish-peroxidase-conjugated anti-mouse IgG or horseradish-peroxidase-conjugated anti-rabbit IgG secondary antibodies (1:10000, DAKO Cytomation, Glostrup, Denmark). Peroxidase activity was visualized with chemiluminescence reaction (Western Lightning, Perkin Elmer, Tokyo, Japan) coupled with film exposure.

Measurement of acoustic startle and prepulse inhibition (PPI)

Acoustic startle and prepulse inhibition (PPI) responses were measured in a startle chamber (SR-Lab Systems, San Diego Instruments, San Diego, CA, USA) adapted for rats (Swerdlow and Geyer 1998; Swerdlow et al. 2001). This paradigm was used to assess startle amplitude, habituation and PPI response with acoustic stimuli of 120 dB, a single prepulse interval (100 msec), and three different prepulse intensities [5, 10, and 15 dB above background noise (white noise, 70 dB)]. Each rat was placed in the startle chamber and initially acclimatized for 5 min with background noise alone. The rat was then subjected to 50 startle trials, each trial consisting of one of five conditions: (i) a 40-msec 120-dB noise burst presented alone (S); (ii–iv) a 40-msec 120-dB noise burst 100 msec after a prepulse (20-msec noise burst) at either 5, 10, or 15 dB above background noise (i.e., 75-, 80-, or 85-dB prepulse, respectively); or (v) no stimulus (N; background noise alone). The last condition was used to measure baseline movement in the chamber. In PPI test, these 5 trial types (i–v) were each repeated 8 times in a pseudorandom order, resulting in 40 total trials. Each trial type was presented once within a block of five trials. At the beginning and end of the PPI test, five consecutive trials of (i) were presented to assess habituation during the session. The inter-trial interval was 15 sec. Analysis of PPI was based on the mean of the eight trials for each trial type. The percentage PPI of the startle response was calculated as:

$$\mathrm{PPI} = 100 - \frac{(\text{startle response on prepulse} - \text{pulse stimulus trials} - \text{no stimulus trials}) \times 100}{\text{pulse-alone trials} - \text{no stimulus trials}}$$

Locomotor activity

We measured locomotor activity in a novel environment using a large size of behavioral chamber as described previously (Futamura et al. 2003). Each rat was placed in an open field box (45 cm length × 45 cm width × 30 cm height, MED Associates, St. Albans, VA, USA) under a moderate light level (400 Lx). Line crossings and rearing counts were measured by photo-beam sensors (25 mm intervals for horizontal axis and 150 mm for vertical axis) for 60 min.

Statistical analysis

Results are expressed as means ± SEM. Statistical differences were determined by analysis of variance (ANOVA) as well as by analysis of covariance (ANCOVA). When univariate data were obtained only from two groups, a two-tailed *t*-test was used for comparison. Behavioral scores were initially analyzed using multiple ANOVA with EGF treatment (two levels), emodin administration (two or four levels) as a between-subject factors and prepulse magnitude (three levels) as a within-subject factor. Interaction of a within-subject factor with between-subject factors was estimated by ANCOVA. When the initial analyses yielded significant factorial interaction, the data were separated to avoid the interaction for the final analyses. Subsequently, a Fisher's LSD test was applied to absolute behavioral values as a *post hoc* test of multiple comparisons. A P value less than 0.05 was regarded as statistically significant. Statistical analysis was performed using the SPSS software (version 11.5). N values in parentheses represent the number of animals used.

Results

Effects of emodin oral administration on startle response and prepulse inhibition

Neurobehavioral impairments were induced in rats with the inflammatory cytokine EGF as described previously (Futamura et al. 2003; Mizuno et al. 2004; Tohmi et al. 2005). EGF or cytochrome c (a control compound for EGF) was administered daily to littermates of neonatal rats (PND2, $n=5$ for each group) for 9 days. At 8 weeks postnatal ($n=5$ each), vehicle or various doses of emodin (5, 15 and 50 mg/kg/day) were given daily (p.o.) to rats for 7 days. One day after the last emodin administration, startle responses to 120-dB tone and prepulse inhibition with 85-dB tones were monitored to estimate the effective dose of emodin. Two-way ANOVA with a between subject factor of neonatal EGF treatment (EGF and cytochrome c) and emodin dose (4 levels) revealed that neonatal EGF treatment exhibited a significant main effect on startle response [$F(1,32)=9.65$, $P=0.039$]. The main effect of emodin dose was not significant [$F(3,32)=0.91$, $P=0.45$] without interaction [$F(3,32)=0.272$, $P=0.85$] (Fig. 1A). In contrast, PPI levels were significantly increased by emodin administration in a dose-dependent manner. Repeated ANOVA with between subject factors of treatment (EGF and cytochrome c) and emodin dose (0, 5, 15, and 50 mg/kg/day) (Fig. 1B) revealed that there were significant main effects of EGF treatment [$F(1,32)=40.8$, $P<0.001$] and emodin dose [$F(3,32)=4.98$, $P=0.006$] with a significant interaction between EGF treatment and emodin dose [$F(3,32)=3.82$, $P=0.019$]. *Post-hoc* analysis indicated that the 15 and 50 mg/kg dose of emodin significantly elevated PPI levels of EGF-treated rats in comparison to the levels of EGF-treated rats not receiving emodin. In contrast, cytochrome c-treated group (control)

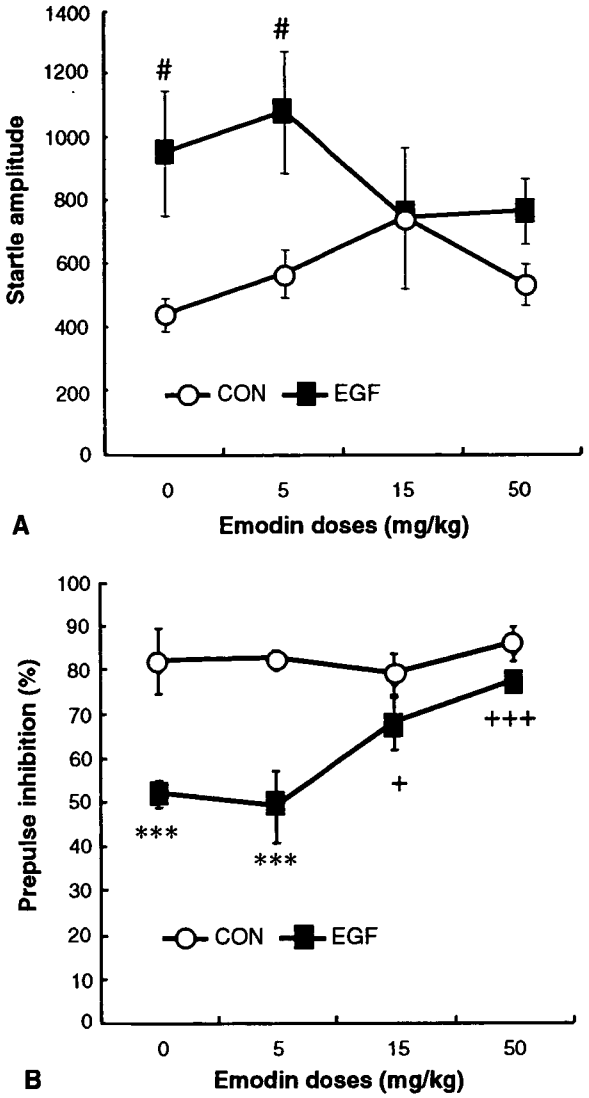

Fig. 1. Emodin dose dependency of PPI recovery in the EGF model. Different doses of emodin (0, 5, 15, and 50 mg/kg/day) were orally administered for 7 days to adult male rats that had been treated with EGF (*closed square*) or cytochrome c (*open circle*) as neonates. (A) The magnitude of pulse-alone startle (120 dB) was plotted against individual emodin doses. Values indicate means ± SEM ($n=5$ each). (B) Prepulse inhibition (PPI) with an 85 dB prepulse stimuli was measured and compared between doses. $^{***}P<0.001$, compared with cytochrome c-treated controls, and $^{+}P<0.05$, $^{+++}P<0.001$, compared with the EGF-treated group not receiving emodin, both by Fisher LSD. $^{\#}$The startle difference between EGF-treated and cytochrome c-treated groups were marginal at the zero and 5 mg/kg doses of emodin (both $P=0.057$) by Fisher LSD

failed to react with emodin. Accordingly, we detected significant differences between EGF-treated and cytochrome c-treated (control) groups at the doses of 0 and 5 mg/kg emodin, but not at higher doses. However, there were no

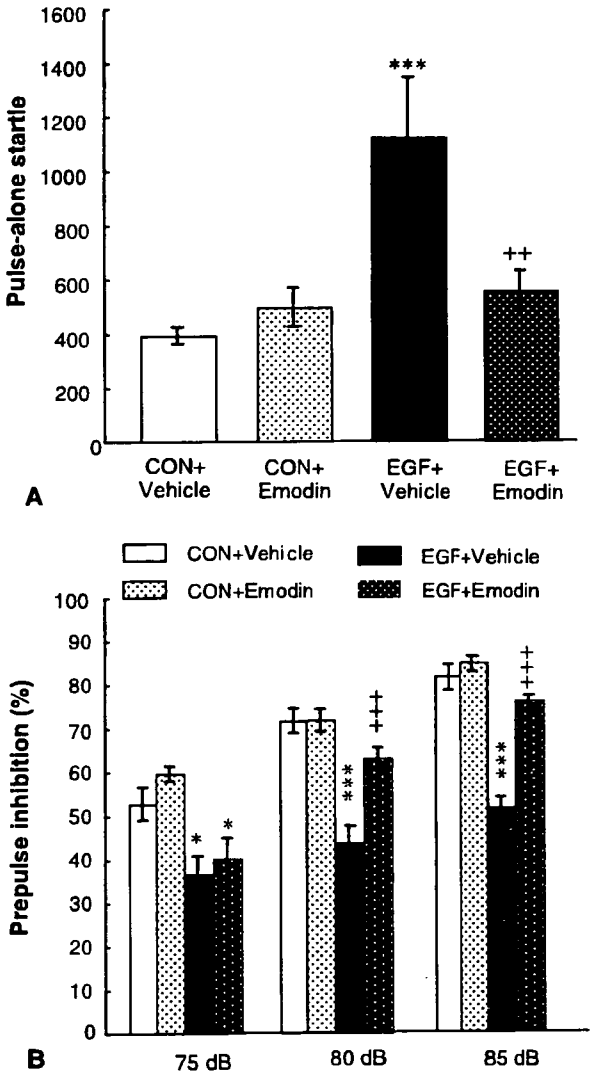

Fig. 2. Effects of subchronic emodin administration on the PPI deficits and startle responses of rats receiving EGF as neonates. Adult male rats were given an emodin emulsion or vehicle orally (10% lecithin) daily for 7 days. One day after the last dose of emodin, pulse-alone startle response to a 120-dB tone (A) and the percentage PPI with 75, 80, and 85 dB prepulse stimuli (B) were measured. Open and black bars represent cytochrome c-treated controls (*CON*) and EGF-treated rats (*EGF*) that received vehicle orally. Black dotted and white dotted bars represent cytochrome c-treated controls and EGF-treated rats that received emodin orally. Bar indicates mean ± SEM for each prepulse intensity ($n = 14$ each). $^{*}P < 0.05$, $^{***}P < 0.001$, compared with cytochrome c-treated controls, and $^{++}P < 0.01$, $^{+++}P < 0.001$, compared with EGF-treated controls that did not receive emodin at the same prepulse intensity, both by Fisher LSD

significant effects on PPI for other prepulse intensities (data not shown).

To confirm the results of this preliminary dose response study for emodin, we prepared four larger groups of animals that had been treated with EGF or cytochrome c as neonates and given either vehicle or emodin subchronically (50 mg/kg) as adults ($n = 14$ for each group) (Fig. 2). Pulse-alone startle responses (120 dB) were significantly altered by neonatal EGF treatment [$F(1,52) = 11.1$, $P = 0.016$, ANOVA] and marginally by emodin administration [$F(1,52) = 3.77$, $P = 0.058$, ANOVA] with a significant interaction between EGF treatment and emodin administration [$F(1,52) = 7.87$, $P = 0.007$, ANOVA] (Fig. 2A). *Post-hoc* analysis revealed that emodin administration specifically suppressed the pulse-alone startle increased by neonatal EGF treatment.

Emodin ameliorated the abnormality in prepulse inhibition (Fig. 2B). Three-way ANOVA with between subject factors of treatment (EGF and cytochrome c) and emodin administration (emodin and vehicle) and a within subject factor of prepulse intensities (75, 80, and 85 dB) revealed significant main effects of EGF treatment [$F(1,52) = 63.1$, $P < 0.001$] and emodin administration [$F(1,51) = 16.6$, $P < 0.001$], and a significant interaction between EGF treatment and emodin administration [$F(2,104) = 7.13$, $P = 0.010$]. We interpret from these data that emodin had a differential effect on EGF-treated and cytochrome c-treated animals. The effects of emodin were separately analyzed in either the EGF-treated or the cytochrome c-treated group. In the EGF-treated group, there was a significant effect of emodin administration on PPI [$F(1,26) = 17.5$, $P < 0.001$, repeated ANOVA] with an emodin × prepulse interaction [$F(2,52) = 9.86$, $P < 0.001$]. *Post-hoc* analysis detected significant effects of emodin administration for 80- and 85-dB prepulses. In contrast, emodin did not have an effect on PPI in the cytochrome c-treated group [$F(1,26) = 1.40$, $P = 0.25$, repeated ANOVA].

As emodin administration affected the pulse-alone startle and specifically PPI levels for higher prepulse stimuli, interpreting these results required detailed analysis (Swerdlow et al. 2001). To test the possibility that the increase in pulse-alone startle responses might promote the decrease in PPI, individual data for EGF-treated rats were re-analyzed by the Peason's correlation analysis followed by ANCOVA (Cadenhead et al. 1993) (Fig. 3). When the percent PPI levels for 85-dB prepulse were plotted versus the magnitude of the pulse-alone startle for each animal of the EGF-treated group, there was weak correlation between these values in vehicle-given rats ($R = -0.60$, $P = 0.023$ for vehicle and $R = +0.185$, $P = 0.54$ for emodin). This suggests that there was a weak contribution of the increase in pulse-alone startle to the reduction in PPI. As there was no significant difference in slope of the line for the vehicle and EGF-treated groups [$F(3,24) = 1.99$, $P = 0.17$], therefore, we re-valuated pure effects of emodin on PPI while assum-

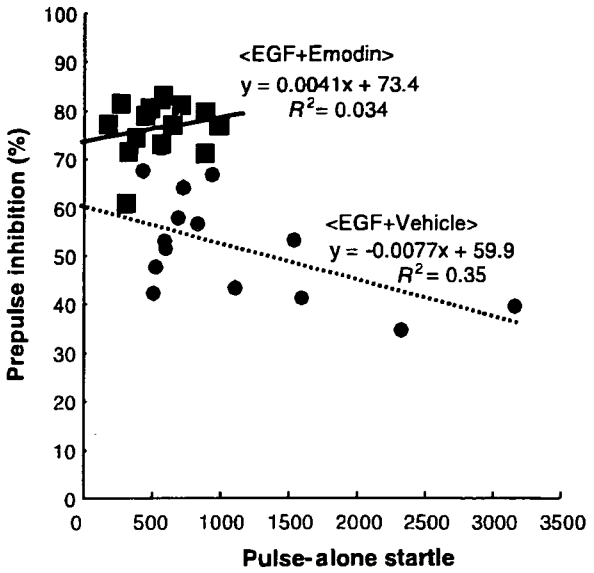

Fig. 3. The effects of emodin on the correlation between PPI and pulse-alone startle. Percentage of PPI obtained with an 85 dB prepulse was plotted against the pulse-alone startle for rats treated with EGF as neonates. The data correspond to Fig. 2. *Circles* represent PPI levels of individual EGF-treated rats given vehicle orally for one week and squares represent those of EGF-treated rats receiving emodin. Emodin administration did not significantly change the slope of the regression curves

ing that the pulse-alone startle influenced the levels of PPI. ANCOVA with the pulse-alone value as a covariate revealed that the group main effect of EGF treatment on absolute PPI retained significance [$F(1,24) = 42.2$, $P < 0.001$, ANCOVA]. Similar statistical results were obtained for the data obtained with an 80-dB prepulse [$F(1,25) = 10.1$, $P = 0.003$]. Thus, we conclude that emodin ameliorates the PPI deficits irrespective of its effect on pulse-alone startle.

Acute effects of emodin on startle response and prepulse inhibition

We also examined the immediate effect of emodin administration on prepulse inhibition (Fig. 4). Vehicle or emodin (50 mg/kg) was orally given to rats. Three hours after administration, startle responses were monitored in the presence and absence of the prepulse stimuli and PPI levels were calculated. Two-way ANOVA for pulse-alone startle revealed that there were no significant effects of emodin administration [$F(1,16) = 0.113$, $P = 0.74$] without interaction [$F(1,16) = 2.07$, $P = 0.17$] (Fig. 4A). Acute administration of emodin failed to mimic the subchronic effects. There was no significant main effect of emodin administration [$F(1,16) = 0.070$, $P = 0.79$] or interaction [$F(1,16) = 1.29$,

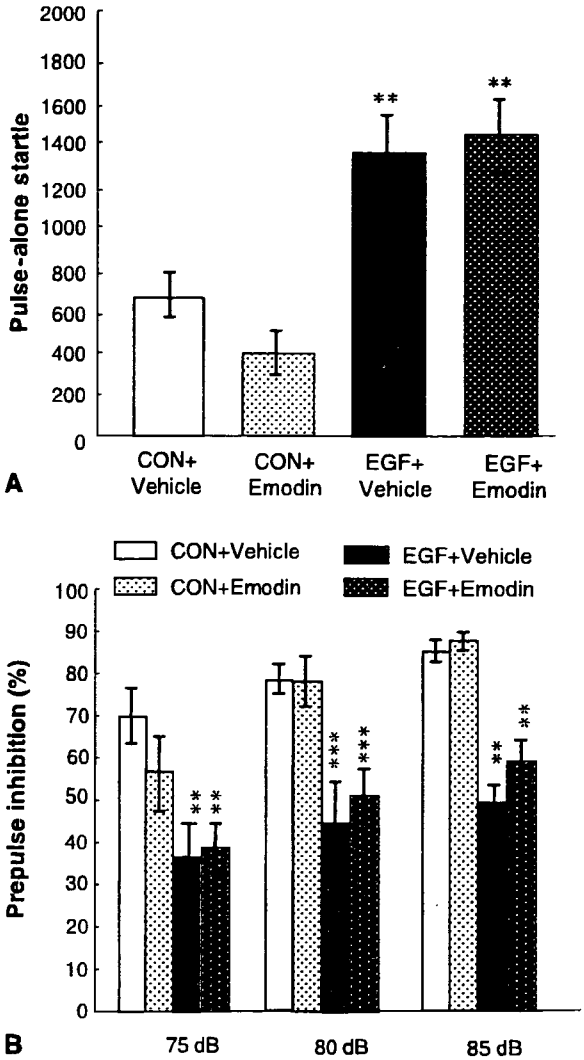

Fig. 4. Acute effects of emodin administration on pulse-alone startle and PPI in the EGF model. Adult male rats were given emodin emulsion or vehicle (10% lecithin) orally. Three hours after administration, pulse-alone startle response to a 120 dB tone (**A**) and percentage PPI with 75, 80 and 85 dB prepulse stimuli (**B**) was measured. Open and black bars represent cytochrome c-treated controls (*CON*) and EGF-treated rats (*EGF*) that received vehicle orally. Black dotted and white dotted bars represent cytochrome c-treated controls and EGF-treated rats that received emodin orally. *Bar* indicates mean ± SEM for each prepulse intensity ($n = 14$ each). $^{**}P < 0.01$, $^{***}P < 0.001$, compared with cytochrome c-treated controls at the same prepulse intensity by Fisher LSD

$P = 0.28$] (Fig. 4B). The present result suggests that repeated administration of emodin is required to improve the PPI deficit induced by neonatal EGF treatment.

Side effects of subchronic emodin administration

Anthraquinone derivatives such as emodin are used as mild purgatives (Nakajima et al. 1985). We examined whether

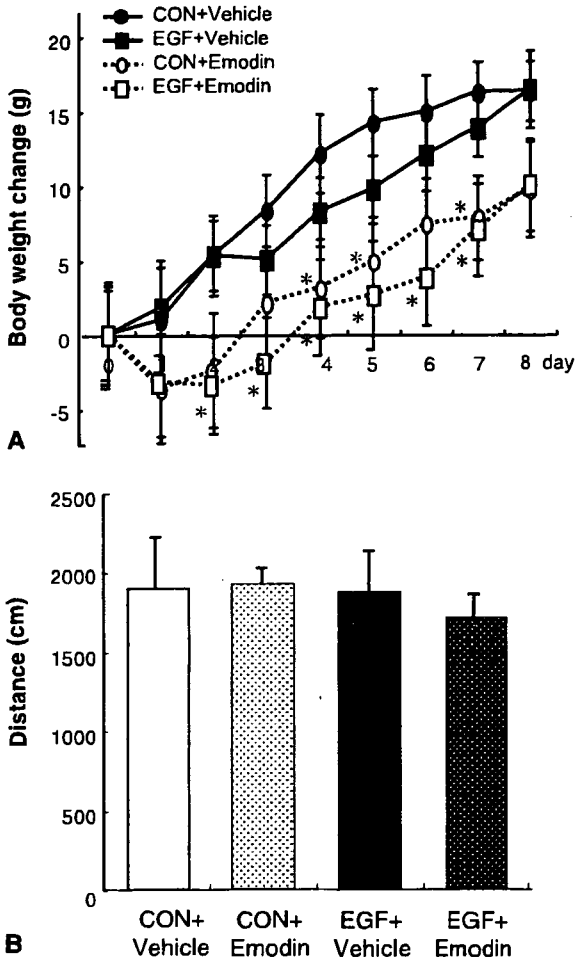

Fig. 5. The effects of emodin on body weight change and locomotor activity. (A) Body weight changes of cytochrome c-treated controls (*CON*) and EGF-treated rats (*EGF*) was determined before emodin administration (day 0), during emodin administration (days 1–7) and one day after administration (day 8) and compared to vehicle-administered controls. Animals were all males and body weight changes from day 0 were monitored before the behavioral tests. Weight gain by emodin-administered rats improved significantly during late sessions. (B) Horizontal movement was monitored for 1 h in a novel environment. Total number of crossings of infrared beams (25 mm intervals) was measured. Bar indicates mean ± SEM ($n = 10$ each, all males). $^*P < 0.05$, compared to cytochrome c-treated controls not receiving emodin (CON + Vehicle) at each day by Fisher LSD. *Note* To minimize acute effects of emodin administration, body weight was measured just before emodin administration

the behavioral effects associated with emodin treatment might be a consequence of emodin-induced defecation. During daily emodin administration, body weight changes were monitored and plotted (Fig. 5A). Emodin significantly reduced body weight one day after the first administration. However, from the third administration onward there were similar positive weight gains in the emodin-treated groups and the untreated groups. The slopes of the regression curves for the overall body weight changes were indistinguishable among four groups after day 3 [$F(1,116) = 0.0004$–0.84, $P = 0.36$–0.99, ANCOVA].

We also monitored locomotor activity one day after the last emodin administration (Fig. 5B) when we measured startle responses and PPI. Two way ANOVA for horizontal movements revealed that there were no significant main effects of EGF treatment [$F(1,16) = 0.370$, $P = 0.55$] nor emodin administration [$F(1,16) = 0.113$, $P = 0.74$] without interaction [$F(1,16) = 0.219$, $P = 0.647$]. Similarly, emodin had no influence on vertical movements [$F(1,16) = 0.439$, $P = 0.52$] (data not shown).

Effects of sennoside on startle responses and prepulse inhibition

To determine whether the effects on PPI are common for all anthraquinone derivatives, we also examined the effect of another anthraquinone derivative, sennoside A. Sennoside A promotes defecation and is popularly prescribed for constipation (Nakajima et al. 1985; Leng-Peschlow 1993; Yamaguchi et al. 1993). Sennoside A (50 mg/kg) was given orally to rats for 7 days and startle responses were measured in the absence and presence of prepulse stimuli. In this experiment, we prepared only two groups of rats treated with EGF and cytochrome c as neonates and evaluated the effects of sennoside A at the adult stage, comparing data before and after sennoside administration (Fig. 6). Two way ANOVA for pulse-alone startle detected a significant main effect of EGF treatment [$F(1,16) = 38.7$, $P < 0.001$] but no effect for sennoside administration [$F(1,16) = 1.35$, $P = 0.26$] nor an interaction [$F(1,16) = 0.489$, $P = 0.50$] (Fig. 6A). Three-way ANOVA for PPI revealed that there were no significant main effects of sennoside [$F(1,16) = 0.577$, $P = 0.46$] without interaction [$F(1,16) = 0.282$, $P = 0.60$], although EGF treatment significantly reduced PPI levels [$F(1,16) = 95.2$, $P < 0.001$] (Fig. 6B). During subchronic administration of sennoside net body weight gain was little or negative (1.6 ± 2.8 g).

Influences of emodin on EGF receptor activation in neuronal culture

Emodin suppresses growth of HER-2/neu-overexpressing breast cancer cells (Zhang et al. 1998, 1999). We monitored the effects of emodin and sennoside on receptor phosphorylation of ErbB1 and ErbB2 in primary cortical cultures (Fig. 7). Neuronal cultures were pre-treated with various doses of emodin (0–300 μM) or sennoside A (0–300 μM) and challenged with EGF (2 ng/ml). In the absence of emodin

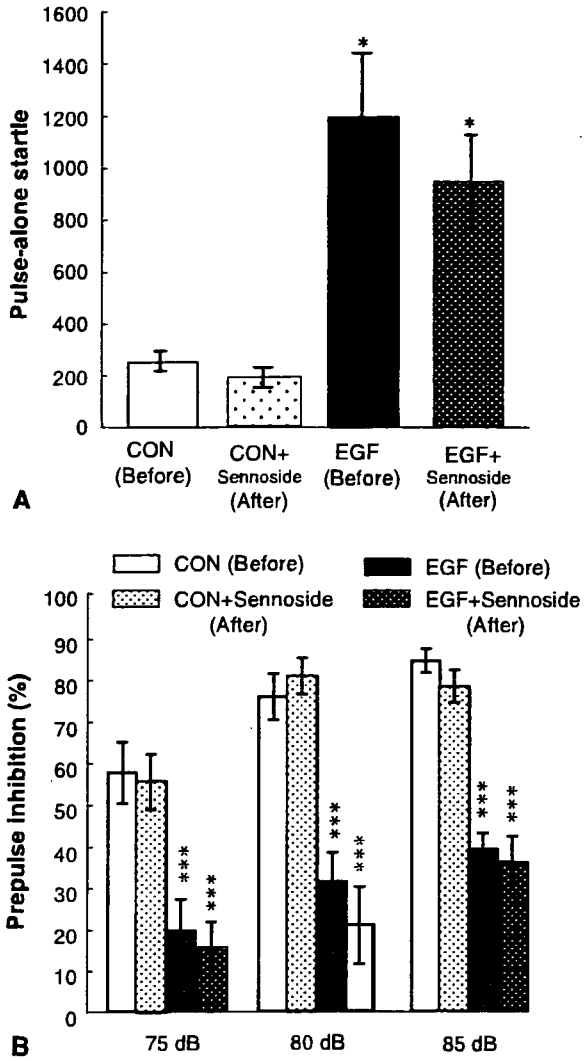

Fig. 6. Effects of subchronic sennoside administration on pulse-alone startle and PPI. Adult rats (male and female) treated with EGF or cytochrome c (control, *CON*) received sennoside (50 mg/kg/day) daily for 7 days. Pulse alone-startle responses (120 dB) and percentage PPI of the acoustic startle response with 75, 80 and 85 dB prepulse stimuli were measured one day before the first sennoside administration (*Before*) and after the last dose of sennoside (*After*). *Open* and *black bars* represent values of cytochrome c-treated and EGF-treated rats before sennoside administration. Black dotted and white dotted bars represent cytochrome c-treated controls and EGF-treated rats after receiving sennoside, respectively. Bar indicate mean ± SEM ($n = 10$ each). $^{*}P < 0.05$, $^{***}P < 0.001$, compared with cytochrome c-treated controls not receiving sennoside by Fisher LSD. *Note* In contrast to the other experiments, both male and female rats were used only in this experiment. There was no significant difference in PPI between male and female rats ($F = 0.005$, $P = 0.946$)

there was a marked phosphorylation of ErbB1 and ErbB2 following EGF challenge. Increasing amounts of emodin attenuated the phosphorylation of ErbB1 and ErbB2 in a dose-dependent manner, whereas the other anthraquinone

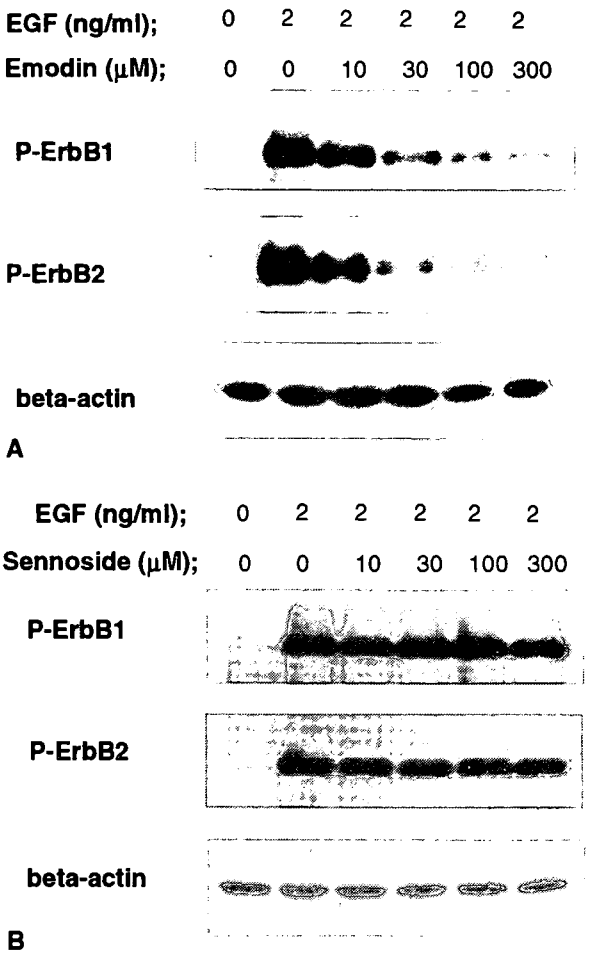

Fig. 7. Acute effects of emodin on ErbB1 and ErbB2 activation in culture. Neocortical neurons were prepared from embryonic rats and grown for 6 days. Cultures were pretreated with various concentration of (**A**) emodin (0–300 μM) or (**B**) sennoside A (0–300 μM) and then challenged to EGF (2 ng/ml final) for 5 min. Phosphorylation of ErbB1 and ErbB2 was monitored by immunoblotting with their antibodies. Immunoreactivity for beta-actin was used as a control

derivative, sennoside A, failed to inhibit the receptor activation. Therefore, the observed behavioral effects of emodin might be ascribed to its inhibitory action on EGF signaling.

Discussion

EGF is an inflammatory cytokine that activates NF-kappaB signaling and induces prostaglandin synthesis (Ackerman et al. 2004; Slice et al. 2005). Abnormal EGF signaling has been implicated in schizophrenia neuropathology (Futamura et al. 2002), consistent with the neuroinflammatory hypothesis of schizophrenia (Muller et al. 2000; Nawa and Takei 2006). As anthraquinone compounds purified from natural herbal extracts inhibit ErbB2-dependent can-

cer proliferation, we tested if the anthraquinone compounds emodin and sennoside A ameliorate EGF-induced behavioral deficits (Futamura et al. 2003). We examined the pharmacological effects of these anthraquinone derivatives on prepulse inhibition and EGF receptor signaling. Subchronic administration of emodin (50 mg/kg/day) ameliorated EGF-triggered behavioral deficits in the acoustic startle reaction as well as in PPI, although acute administration had no effect on PPI. In addition, subchronic administration of emodin attenuated weight gain but had no effect on locomotor activity. Emodin, but not sennoside, attenuated EGF-triggered activation of ErbB1 and ErbB2 in primary neuronal cultures. Sennoside A did not mimic the behavioral effects of emodin but it had a greater effect on limiting weight gain. This gain during sennoside treatment (1.6 ± 2.8 g) was significantly smaller than that during emodin administration (9.8 ± 2.6 g gain). Therefore, it is less likely that physical impact of the emodin's purgative activity was not causative for the effects of emodin on behavior. From these findings, we propose that oral administration of emodin can improve neurobehavioral deficits associated with EGF signaling.

Emodin used in the present study was purified from rhubarb. To avoid detecting the biological activity of contaminating agent(s), we used emodin from three different sources. The dose-responses were obtained with emodin from Sigma Chemical Inc. (90% purity). In the acoustic startle experiment, two lots of emodin from Tokyo Chemical Industry Inc. (96, 99% purity) were used. As all lots of emodin tested consistently produced behavioral effects, we conclude that emodin is the causative agent responsible for the behavioral results. However, emodin decreased acoustic startle responses, the explanation of prepulse inhibition may be complex. Although statistical analysis with ANCOVA revealed the dissociation between PPI and startle responses for EGF-treated animals, we cannot fully rule out the possibility that emodin-triggered decrease in startle amplitudes contributed to the normalization of PPI.

The pharmacological activity of emodin is distinct from that of the atypical antipsychotic clozapine. Subchronic treatment with clozapine does not normalize the higher pulse-alone startle amplitudes of the EGF-treated rats but significantly improves their PPI score (Futamura et al. 2003). The antipsychotic potency of clozapine appears to be lower than that of emodin, however. Clozapine does not fully normalize PPI of the present EGF model to a control level whereas emodin does (Futamura et al. 2003).

There are many anthraquinone derivatives that have been extracted and purified from numerous plants (Wang et al. 2001; Huang et al. 2007). These compounds include emodin, sennoside, chrysophanol, aloe-emodin, physcion and rheum. Extracts from these Chinese herbs containing these compound are most famous for their promoting defecation but may also possess additional activities, including affecting various psychiatric conditions. For example, extracts from mixed Chinese herbs including rhubarb rhizome (the major source of emodin) have been proposed to be anxiolytic. A recent clinical study examining 67 schizophrenia patients reported the effectiveness of combined therapy of a leech-rhubarb mixture and antipsychotic drug (Zhu et al. 1996). A double blind study in Germany demonstrated that chronic administration (12 weeks) of a rhubarb extract decreased the anxiety of treated individuals on Hamilton Anxiety Scale to one third (Kaszkin-Bettag et al. 2007). These findings are consistent with rhubarb containing a compound(s) that have a psychopharmacological effect like an antipsychotic. The present study suggests that emodin might be one of these active ingredients.

Here we tested the potential anti-psychotic activity of emodin and its derivative in this schizophrenia model, assuming that emodin or its metabolites penetrates the blood-brain barrier and acts on brain neurons. Our previous studies demonstrate that EGF signaling is upregulated in schizophrenia patients as well as in the present rodent schizophrenia model established with neonatal EGF challenge (Futamura et al. 2002; Tohmi et al. 2005; Nawa and Mizuno 2006). Although emodin actions in the brain remain to be characterized, its effects on attenuating behavioral deficits emerged after its subchronic administration. In contrast to the in vivo effects, acute application of a low dose of emodin (30 μM) was enough to attenuate the receptor activation of ErbB1 and ErbB2 following EGF challenge in primary neuronal cultures. We can speculate the reason why behavioral effects of emodin require subchronic administration of emodin: efficacy of emodin penetration through the blood-brain barrier is low. Alternatively, emodin effects on behaviors through ErbB signaling involve structural alterations of the brain circuits.

In addition to the emodin effect on sensorimotor gating, our preliminary study indicates that this compound is effective to ameliorate methamphetamine sensitization but not abnormal social interaction of the present EGF model (unpublished data). In this context, emodin might have a limited anti-psychotic action against positive symptoms. However, emodin can be given orally and shows only the modest and time-limited effect on defecation. Accordingly, we propose that emodin might be an interesting pro-drug for schizophrenia medication targeting ErbB signaling.

Acknowledgments

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第103回日本精神神経学会総会

シンポジウム

脳由来神経栄養因子遺伝子多型とストレス脆弱性

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はじめに

近年，脳由来神経栄養因子（brain-derived neurotrophic factor：BDNF）は，種々の精神・神経疾患の病態生理や向精神薬の作用機序における鍵分子の1つとして注目されている．BDNFは，神経成長因子（nerve growth factor：NGF）やニューロトロフィン3，ニューロトロフィン4/5などと共にニューロトロフィン・ファミリーに属する神経栄養因子の1つである．これらの神経栄養因子は，中枢神経系の成長，分化，維持，可塑性などにおいて重要な役割を果たしている．ニューロトロフィンは図1のように，それぞれが特異的な高親和性受容体（TrkA, TrkB, TrkC）をもつと同時に，全てのニューロトロフィンに共通の低親和性受容体p75がある．興味深いことに，Trk受容体はチロシンキナーゼ・ドメインをもち上記のような細胞栄養作用をもつが，p75は腫瘍壊死因子スーパーファミリーに属し，death domainを所有し，アポトーシスなどにおいて重要な働きをなすとされる．さらに，ニューロトロフィンは前駆体タンパクからプロセシングを受けて成熟タンパクとなるが，この前駆体タンパクは，p75に対する親和性が高い（図1）．つまり，BDNFはプロセシングや受容体への親和性によって相反する作用をもたらすいわば“諸刃の刃”である．

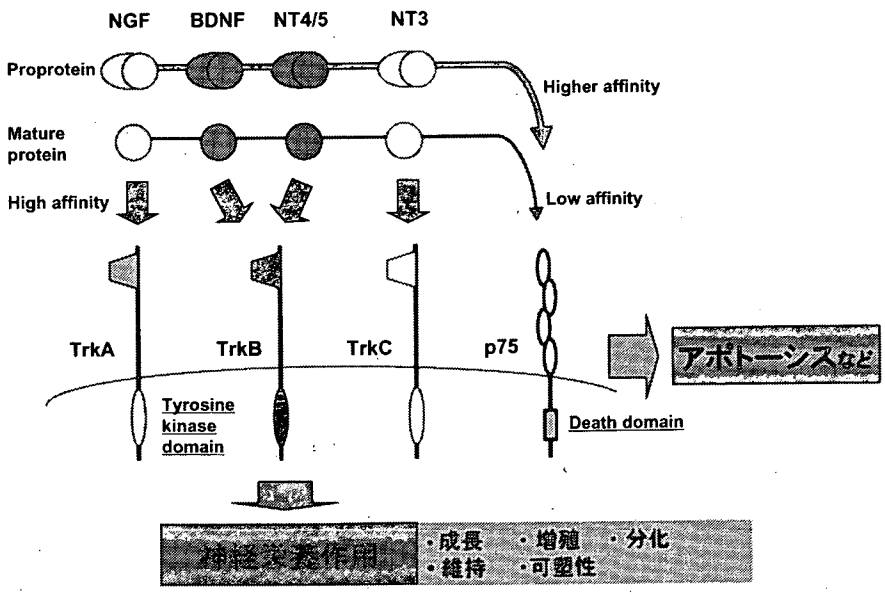

図1　ニューロトロフィンと受容体

BDNFと精神疾患

BDNFが統合失調症や気分障害などの機能性精神疾患において重要な働きをしていることに関しては，患者死後脳や血中濃度に関する所見，統合失調症や気分障害の動物モデルにおける発現変化，向精神薬による発現の変化など，種々の点からのエビデンスが集っている．

統合失調症死後脳における発現変化を報告したのは，新潟大学のTakahashiら[22]がおそらく最初であり，統合失調症患者死後脳では，前帯状回や海馬ではBDNFの発現は上昇しているが，前頭前野では低下しており，TrkBの発現は海馬でも前頭前野でも低下していたと報告している．その後の報告では，前頭前野におけるBDNFとTrkBの発現低下はかなり一致した所見であり[8,25]，海馬については低下しているという報告もある[12]．統合失調症患者におけるBDNFの血中濃度に関する報告も新潟大によるものがおそらく最初であり，統合失調症患者（服薬中）はコントロールと比較して，BDNF濃度が低下していると報告した[24]．その後の研究では低下しているという報告と有意差を認めなかったという報告があり一定しないが，BDNFが上昇していたという報告はない．なお，BDNFは血小板中に多く含まれていることもあり，血中濃度がどの程度脳内のBDNF濃度を反映しているかについてはよくわかっていない．

統合失調症の動物モデルでは，例えば，ラット出生直後の腹側海馬障害モデル[17]では，前頭前野や海馬歯状回においてBDNFレベルの低下が見られ，妊娠ラットにストレスを加えて作製したモデル（仔ラット）では，前頭前野や線条体においてBDNFの発現が低下していた[6]．

抗精神病薬によるBDNFの発現の変化も多数研究されており，結果は必ずしも一致してないが，ハロペリドールや高用量のリスペリドンでは海馬のBDNF mRNAを低下させるが[3]，クエチアピンやオランザピンなどの非定型的抗精神病薬では，BDNFのmRNAを増加させるなど[20,21]，D_2受容体への結合が強い薬物は低下させる傾向，5-HT_{2a}受容体への結合が強いものは増加させる傾向がある可能性がある．

気分障害についての詳細は省くが，うつ病で自殺した者の死後脳では海馬においてBDNFが減少しており，未服薬のうつ病患者では血中BDNF濃度が低下し，抗うつ薬の投与によって濃度が上昇に転じるという報告が多い[4]．動物実験では，拘束ストレスなどの慢性的ストレス負荷やグルココルチコイドの投与によってBDNFが減少し，副腎摘除によってグルココルチコイドの産生を抑えると，BDNFは増加するという結果が多い[4]．なお，著者らは抗うつ薬はBDNFの発現を増加させるだけでなく，BDNFの機能を増強させる作用をもつことを最近報告した[26]．

以上のように，BDNFの機能異常が統合失調症などの精神疾患の病態や治療において重要である可能性を示唆するエビデンスが増えているが，BDNFにはいくつかの遺伝子多型が報告されており，それに基づく機能異常が精神疾患の発病脆弱性，ストレス脆弱性に関与している可能性がある．

BDNFの遺伝子多型と統合失調症

図2に示すようにBDNFには3つのよく知られた遺伝子多型があり，精神・神経疾患との関連研究が多数なされている．1つは，アミノ酸置換（Val66Met）を伴う一塩基多型であり，これはヒトBDNF遺伝子が最初にクローニングされた際に塩基配列の違いがあったことから，多型の存在が推定されていた．著者らが最初にBDNF遺伝子の多型スクリーニングを行い，その多型の存在を確認し統合失調症との関連を調べたが，有意な関連は認められなかった[18]．しかし，このアミノ酸置換は遺伝子機能を変化させることが明らかにされ，Met型はVal型に比べて脱分極によって誘導されるBDNFの放出が低下することや，ニューロン内での分泌顆粒への移動が障害されていること，ヒトでMet型をもっている者は，そうでない者と比べて海馬の活性化が低下しており，エピソード記憶の成績が低いことなどが報告され

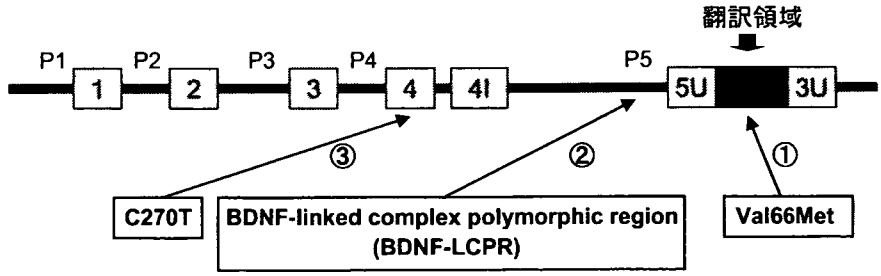

図2　BDNF遺伝子構造と遺伝子多型
遺伝子構造はAoyamaら[2]による．

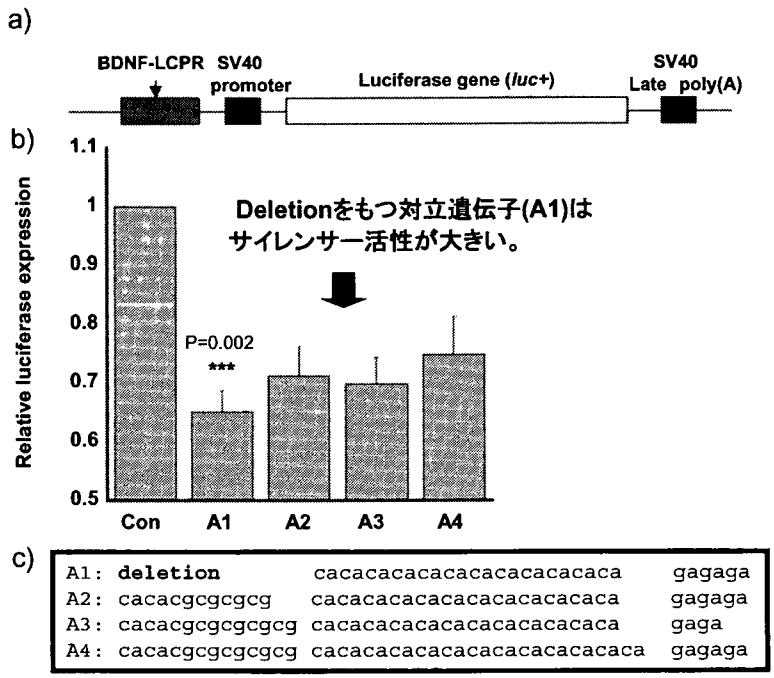

図3　BDNF-LCPRの主要な対立遺伝子と転写活性　（文献19）より）

た[5]．その後，多数の関連研究がなされたが，最近のメタアナリシスの結果では，統合失調症との関連を否定するものが多く，例えば，2955人の統合失調症患者と4035人のコントロールをプールしたメタアナリシスでは，オッズ比は丁度1.00で，有意な関連はなかった（P＝0.944）[11]．

2つめの多型は，翻訳領域を含むエクソン5の5'上流にあるマイクロサテライト多型である．この多型は，当初，2塩基繰り返し配列と報告されていたが，われわれの詳細な解析の結果，3種類の2塩基繰り返し配列がタンデムにつながっており，欠失/挿入なども存在する極めて複雑な多型であることが明らかになり，BDNF-linked complex polymorphic region（BDNF-LCPR）と命名した[19]．さらに対立遺伝子によって転写活性が異なる可能性が示唆され（図3），低活性と関連する対立遺伝子は双極性障害のリスクを高めることを見出した[19]．次に，この多型と統合失調症との関連を調べたところ，低活性と関連すると推定される“del型”対立遺伝子が統合失調症のリスクを高める可能性を示唆する結果を得た[10]．これらの結果は，BDNF-LCPRが転写活性に影

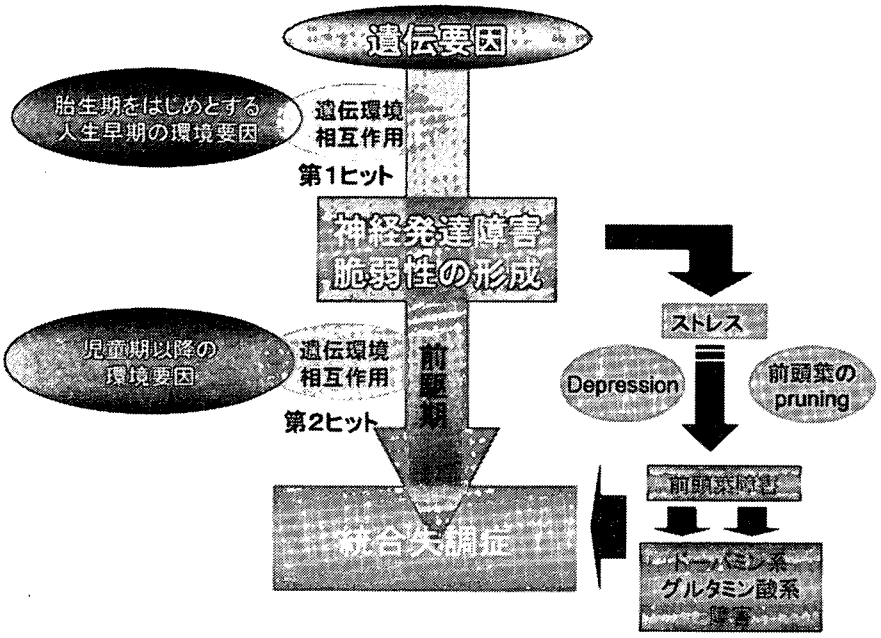

図4　統合失調症の発病スキーマ

響を与えることにより，双極性障害や統合失調症のリスクを高める可能性を示唆する．

3つめの多型は，非翻訳領域にあるC270T多型であり，著者らが最初に見出し，アルツハイマー病や統合失調症との関連を報告した[16,18]．この多型と統合失調症との関連についても多数の追試がなされており，ごく最近のメタアナリシスによれば，T型対立遺伝子は統合失調症のリスクを高めることが示唆されている（オッズ比1.63 95％信頼区間1.01-2.65）[27]．この多型がBDNFの機能に対して影響があるかどうかは不明であるが，Agartzら[1]は，MRIによる脳構造体積との関連を解析し尾状核の体積と関連すると述べている．また，細胞体から伸展するデンドライト数に影響するのではないかという知見が学会発表レベルでなされている．

統合失調症の発病スキーマとBDNF遺伝子多型の関与

上述のようなBDNFの遺伝子多型が，統合失調症のストレス脆弱性にどのように関与するかについて考察してみたい．統合失調症の主な成因仮説には，ドーパミン仮説，グルタミン酸機能低下仮説，神経発達障害仮説，刈り込み（pruning）異常仮説（神経ネットワーク異常仮説）などがあるが，これらを総合的に説明するものとして図4のような発病スキーマを提示する．このスキーマでは，統合失調症の発病を2段階（2ヒット）でとらえる．すなわち，胎生期を含めた人生早期においては低出生体重[13]やインフルエンザなどへのウイルス感染[14]，低酸素などの産科合併症[15]を含む環境要因と遺伝要因との相互作用によって，ストレス脆弱性が形成される（第1ヒット）．そのストレス脆弱性によって，児童期以降にストレスを受けやすくなる，あるいは通常はストレスとならないような出来事が慢性的なストレスとなり，うつ状態を伴う前駆期に至る．なお，最近，統合失調症の前駆症状の研究が進んでおり，統合失調症患者の殆どは幻覚妄想状態になる3〜4年前から抑うつ症状や陰性症状・不安症状が出現することが明らかにされている[7]．この時期では，思春期/青年期の大きなホルモン環境の変化とともにおそらく視床下部-下垂体-副腎系のストレスホルモン（グルココルチコイド）の過剰などによる脳

内の変化が生じており，それが前頭葉の刈り込み(pruning)が完成する時期に重なる．ストレスホルモンなどの脳内変化は遺伝要因との相互作用によって，前頭葉を中心とした神経ネットワークの過剰な脱落などの傷害を与えると考えられる(第2ヒット)．これは，統合失調症を発症したものでは，青年期における前頭葉などの皮質体積の減少が大きいというMRIによる観察[23]によって裏付けられる．ここではメカニズムに関しては省くが，こうした前頭葉を中心とした脳の障害が，ドーパミン系やグルタミン酸系の機能異常をもたらす．

BDNFの遺伝子多型による機能異常は，第1ヒット，第2ヒットの両者に関与すると思われる．すなわち，人生早期の第1ヒットでは，低酸素などの環境リスク下で神経栄養作用をもつBDNFの機能異常があれば，受ける脳の障害がより大きくなると考えられる．また，第2ヒットのストレスを受ける時期でも，BDNFの機能異常があれば，神経ネットワークの脱落がより大きくなると考えられる．統合失調症の発症には上述のように思春期/青年期における慢性ストレスやうつ状態が重要な役割を果たす可能性を考慮すれば，BDNFが統合失調症と気分障害という異種の病気の鍵分子となる点は矛盾なのではなく，むしろ重要なポイントであるといえるだろう．

おわりに

いうまでもなく，統合失調症の遺伝-環境相互作用における遺伝要因は，BDNFだけではない．また，BDNFの遺伝子多型はストレス脆弱性や発病のリスクをある程度高めるだけであり，決定的なものではない．ニューロトロフィン関連でも，BDNF以外にニューロトロフィン3の遺伝子多型[9]などが関連する可能性があるし，そのほか，例えばDISC1やdysbindinのように統合失調症のリスクを高めるものとして有力な種々の遺伝子がある．しかし，BDNFに関する所見は，遺伝子関連解析だけでなく，死後脳，血中濃度，動物モデルなどさまざまな観点から精神神経疾患における鍵分子であることを示唆する所見が集積している．したがって，この分子を1つの鍵分子として統合失調症の病態の解明や新たな治療法の開発を行うことは極めて有効かつ重要なストラテジーであろう．

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