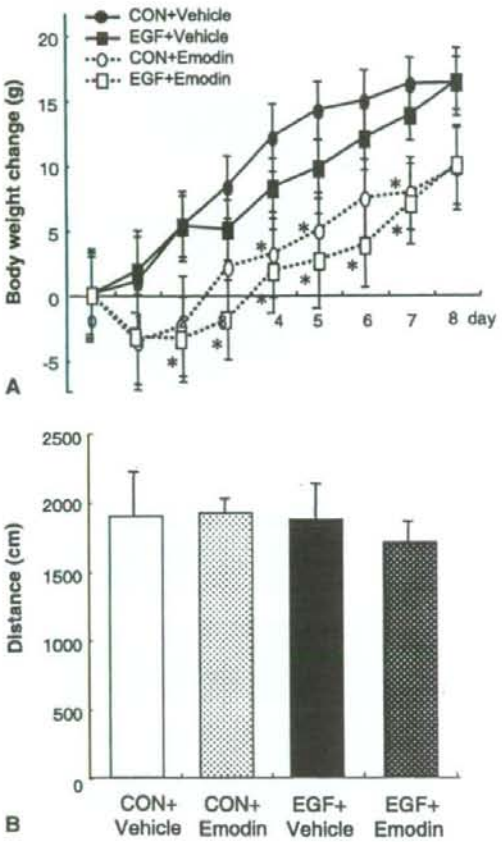

Fig. 5. The effects of emodin on body weight change and locomotor activity. (A) Body weight changes of cytochrome c-treated controls (*CON*) and EGF-treated rats (*EGF*) was determined before emodin administration (day 0), during emodin administration (days 1–7) and one day after administration (day 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

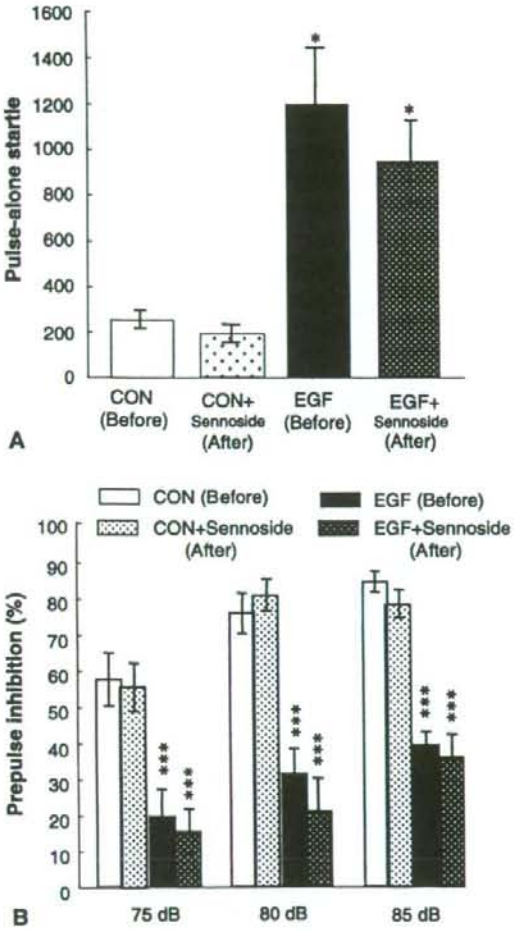

Fig. 6. Effects of subchronic sennoside administration on pulse-alone startle and PPI. Adult rats (male and female) treated with EGF or cytochrome c (control, *CON*) received sennoside (50 mg/kg/day) daily for 7 days. Pulse alone-startle responses (120 dB) and percentage PPI of the acoustic startle response 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 ($F = 0.005$, $P = 0.946$)

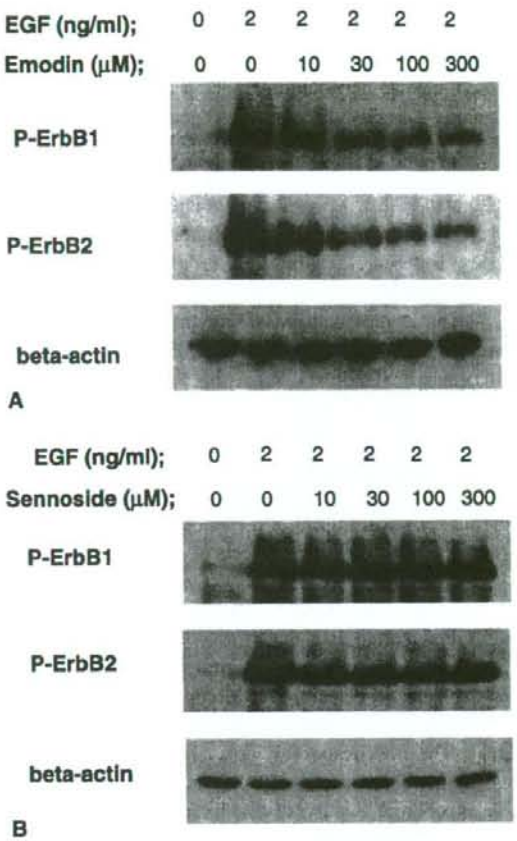

Fig. 7. Acute effects of emodin on ErbB1 and ErbB2 activation in culture. Neocortical neurons were prepared from embryonic rats and grown for 6 days. Cultures were pretreated with various concentration of (**A**) emodin (0–300 μM) or (**B**) sennoside A (0–300 μM) and then challenged to EGF (2 ng/ml 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

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 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.

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Original article

Common behavioral influences of the ErbB1 ligands transforming growth factor alpha and epiregulin administered to mouse neonates

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Abstract

Ligands for epidermal growth factor (EGF) receptor (ErbB1), such as EGF, transforming growth factor alpha (TGFα), and epiregulin, are enriched in body fluids and blood and regulate development of various peripheral organs. It remains however how such circulating polypeptide growth factors influence brain development and function. Here, we performed peripheral injections of TGFα and epiregulin to mouse neonates and evaluated immediate physical and neurochemical development and later behavioral consequences. Subcutaneous administration of TGFα and epiregulin increased phosphorylation of brain ErbB1, suggesting their effects on brain development. Repeated their injections similarly enhanced physical development of eyelid opening and tooth eruption during early postnatal stage and resulted in abnormal behavioral traits in the adult stage. Acoustic startle responses of mice treated with these growth factors as neonates were enhanced and prepulse inhibition was decreased without an apparent correlation between prepulse inhibition level and startle intensity. Locomotor activity and fear-learning performance with tone and context cues were not altered, however. These results suggest that circulating ErbB1 ligands in the periphery of neonates have some common influences on later behavioral traits. Abnormal ErbB1 ligand production at neonatal and potentially prenatal stages might therefore associate with neurodevelopmental disorders such as schizophrenia.

Keywords: EGF; Cytokine; Body weight; Tooth eruption; Eyelid opening; Sensorimotor gating; Animal behavior; Neurodevelopmental disorder

1. Introduction

ErbB1 receptor tyrosine kinase binds to various peptide ligands including epidermal growth factor (EGF) and transforming growth factor alpha (TGFα), heparin-binding EGF-like factor (HB-EGF), amphiregulin, betacellulin, epigen, and epiregulin [1–4]. These ligands are synthesized as membrane-anchored precursors in a large variety of peripheral tissues, such as, kidney, blood cells, placenta, etc., and liberated to urine, blood, and amniotic fluid [5–9]. As all these ligands have a cell mitotic activity and thus are implicated in growth regulation of peripheral organs as well as in cancer progression [9–12]. Our recent studies on brain development indicate that exogenously supplied EGF in the periphery can penetrate the developing brain–blood barrier (BBB) of neonatal rats and mice to perturb phenotypic development of brain neurons [13]. Therefore, such growth

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factors in blood stream have latent but profound impact on brain development during early prenatal and postnatal stages [14]. It is unknown, however, how endogenous growth factors reach the brain to perturb brain development and function.

ErbB1 is expressed in many types of brain cells, but is especially enriched in midbrain dopaminergic neurons as well as neural stem cells [15,16]. Our previous studies suggest that EGF efficiently penetrates the immature blood–brain barrier during early rodent development and later perturbs dopamine-associated animal behaviors, such as sensorimotor gating, social interaction, exploratory motor activity and amphetamine sensitivity [13,17,18]. These findings have indicated that not only EGF but also other ErbB1 ligands in some human fetuses or neonates potentially influence brain development and future psychobehavioral traits. However, it remains to be determined which ErbB1 ligands contribute to the establishment of psychiatric disease or traits.

In the present investigation, we evaluated the biological significance of the ErbB1 ligands, TGFα, and epiregulin, in determining behavioral traits in mice. Mice were subjected mainly to the behavioral tests that are often used to evaluate schizophrenia models. Their effects on behaviors and physical development were compared with the rodent model established by neonatal exposure to EGF [13,17].

2. Materials and methods

2.1. Animal protocols

Four strains of neonatal mice (C57BL/6N, 12 litters total; Nihon Charles River, Yokohama, Japan) were purchased together with their dams. Recombinant human epiregulin (1.0 μg/g; Taisho Pharmaceutical Inc., Ltd, Japan) or TGFα (1.0 μg/g; Peprotech Inc., Ltd, UK) was administered subcutaneously to half of individual litters daily during postnatal days (PND) 2–10. Control littermates of the other half received saline injections. After PND 20, mice were separated according to gender and raised separately (3–4 mice per cage; 13.6L × 20.8W × 11.5H cm). All mice were housed on a 12-h light–dark cycle with free access to food and water. Physical indices such as body weight, eyelid opening, and tooth eruption were monitored daily (17:00–19:00 h). All animal experiments were authorized by the Animal Use and Care Committee of Niigata University and performed during the day cycle (10:00–19:00 h). Animals (TGFα-, epiregulin- or saline-treated mice, $n = 10$–17 per group) were assigned to three behavioral tests to minimize the effects of the preceding behavioral test; locomotor test followed by prepulse inhibition (PPI) and then followed by the contextual conditioning task. Mice were subjected to behavioral tests during PND 56–70 with 3–7-day interval between tests. Both sexes were equally represented. Mice were habituated to experimental rooms, at least, 2–3 h before testing.

2.2. Immunoblot analysis

Using an independent set of animals, TGFα or epiregulin (1.0 μg/g) or saline was injected subcutaneously into mouse pups (PND2) to examine the acute effect on ErbB1 activation in the brain. Brains were removed 15–240 min after injection and homogenized by sonication with 1× sample loading buffer (10 mM Tris–HCl, 150 mM NaCl, 2% SDS, 20 mM NaF, 1 mM Na_3VO_4). To confirm subchronic influences of these factors on brain neurons, alternatively, tissue lysate was prepared from the frontal cortex of mice (PND11) one day after repeated administration (PND2–PND10). Protein (50 μg/lane) was subjected to a 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane. The membrane was probed with antibodies directed against phosphorylated ErbB1 (1:1000; Cell Signaling, Beverly, MA, USA). Alternatively, immunoblots were probed with anti-GluR1 receptor (1:1000), anti-GluR2/3 (1:1000), and anti-β actin (1:1000) (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunoreactivity was visualized with chemiluminescence reaction (Western Lighting, Perkin-Elmer, Tokyo, Japan).

2.3. Analysis of locomotor activity

Exploratory motor activity was measured in a novel environment with the following test equipment under dim light (27L × 27W × 20H cm, MED Associates, St. Albans, VA, USA). Mice were placed in an automated activity monitor equipped with infrared photosensors. Horizontal activity for every 5 min was measured as beam crossings during the initial 60 min and analyzed by using fully automated tracking system (Activity Monitor, Med Associates).

2.4. Measurement of acoustic startle response and prepulse inhibition

Mice were placed in a plastic cylinder and fixed in an automated startle chamber (SR-Lab Systems, San Diego, CA, USA). After a 5-min acclimation period with 70-dB-background noise (white noise), an 75-, 80-, 85-, 90-, 100-, 110-, or 120-dB white noise stimulus (40 ms duration) was given 8 times to each mouse in the same pseudo-random order at 15 s-intervals. Analysis for startle amplitudes was based on the mean of the seven trials (ignoring the first trial) for each trial type.

Using a different set of mice, PPI responses were measured with 120-dB acoustic stimuli combined with four different prepulse intensities. Each mouse was placed

in the startle chamber (SR-Lab) and initially acclimatized for 5 min with background noise alone (70-dB white noise). The mouse was then subjected to 48 startle trials, each trial consisting of one of six conditions: (i) a 40-ms 120-dB noise burst presented alone (S), (ii–v) a 40-ms 120-dB noise burst following prepulses by 100 ms (20-ms noise burst) that were 3, 6, 9, or 12 dB above background noise (i.e., 73-, 76-, 79-, or 82- prepulse, respectively), or (vi) no stimulus (background noise alone), which was used to measure baseline movement in the chamber. These six trial types (i–vi) were each repeated 8 times in a pseudorandom order to give 48 trials. The inter-trial interval was 15 s. Each trial type was presented once within a block of six trials and the order of 48 trial presentations was fixed for all mice. Analysis was based on the mean of the seven trials for each trial type. The percentage PPI of a startle response was calculated as: 100 – [(startle response on prepulse–pulse stimulus trials – no stimulus trials)/(pulse-alone trials – no stimulus trials)]) × 100) [19,20].

2.5. Contextual conditioning

The test paradigm of contextual conditioning was based on a work by Tohmi et al. [17]. Mice were placed in a shock chamber with a stainless steel grid floor (10W × 10D × 10H cm box; Ohara Medical Industry Inc., Tokyo, Japan) for 2 min to monitor baseline movement/freezing and then exposed to 0.8-mA electric shocks (2-s duration, 3 times at an interval of 90 s) all together with 30-s tone cues (60-dB, 10 kHz). One day after conditioning, mice were returned to the same chamber. The time spent freezing was recorded and averaged every 30 s. After 3 h, mice were moved to a different chamber with a flat floor (10W × 10D × 10H cm box) and the time spent freezing was recorded and scored for 3 min before and after the tone cue. Freezing behavior was automatically monitored by a video camera during all sessions and analyzed with the aid of an imaging software (Ohara Medical Industry Inc.).

2.6. Statistical analysis

Results were expressed as means ± SEM. Startle response, PPI, and learning measures were analyzed using ANOVA with repeated measures, followed by a Fisher LSD post hoc test for groups having similar deviations or a Games-Howell post hoc test for groups having different deviations. In repeated measures ANOVA, a between subject factor was treatment (TGF-α/epiregulin and PBS) and a within subject factor was either test session or prepulse intensity of absolute or percentage reduction of startle amplitudes. A Pearson's correlation test between pulse-alone startle responses and PPI levels was performed, followed by ANCOVA with a subject factor of treatment and a covariate of an absolute amount of pulse-alone startle. To quantify immunoreactivity on blots, the densitometry of bands (arbitrary units) was performed and subjected to two-way ANOVA with subject factors of treatment and age or univariate analysis of Student's *t*-test for data at a single age. *N* values represent the number of animals. A p value of less than 0.05 was considered statistically significant. Statistical analysis was performed using the SPSS software (version 11.5; SPSS Japan Inc., Tokyo, Japan).

3. Results

3.1. ErbB1 activation following peripheral injection of TGF-α and epiregulin

We have previously reported that peripheral administration of EGF activates ErbB1 in the neonatal brain, presumably penetrating the immature blood–brain barrier [13,21]. In the present study, we examined whether peripheral administration of the EGF homologs, TGF-α, and epiregulin, similarly triggers ErbB1 phosphorylation in the brain (Fig. 1). A single subcutaneous injection of TGF-α to neonatal C57BL/6N mice immediately increased phosphorylation of ErbB1. There was a marked increase in immunoreactivity for phosphorylated ErbB1 at the 15-min period. The increase was disappeared within 2 h. In contrast to TGF-α, epiregulin produced a more modest and prolonged increase in phosphorylated ErbB1 levels. β-Actin levels (control) were not significantly altered ($F[5,11] = 0.393$, $p = 0.063$ for TGF-α; $F[5,11] = 3.397$, $p = 0.062$ for epiregulin).

We previously found that EGF and TGF-α attenuate the developmental increase of AMPA receptor expression in the neocortex [22,23]. To control the present experimental procedure, we examined whether the administration of epiregulin mimics the effect of TGF-α in neonatal mice (Fig. 2). Epiregulin and TGF-α were daily administered to neonatal mice until PND10 and protein levels for AMPA receptors (GluR1 and GluR2/3) were examined in the neocortex by immunoblotting. The both EGF homologs attenuated the protein expression of GluR2/3 ($F[2,12] = 12.69$, $p = 0.001$ after normalization with actin levels) (Fig. 2B). There was no significant influence of those ErbB1 ligands on GluR1 protein levels, however ($F[2,12] = 0.862$, $p = 0.445$ after normalization with actin levels).

3.2. Effects of TGF-α and epiregulin treatment on physical development of mice

Repeated administration of EGF to mouse newborn pups accelerates eyelid opening and tooth eruption [10]. To compare the peripheral effects of TGF-α and epiregulin, we similarly administered these factors or saline subchronically into mouse pup and monitored these physical indices (Table 1). TGF-α treatment

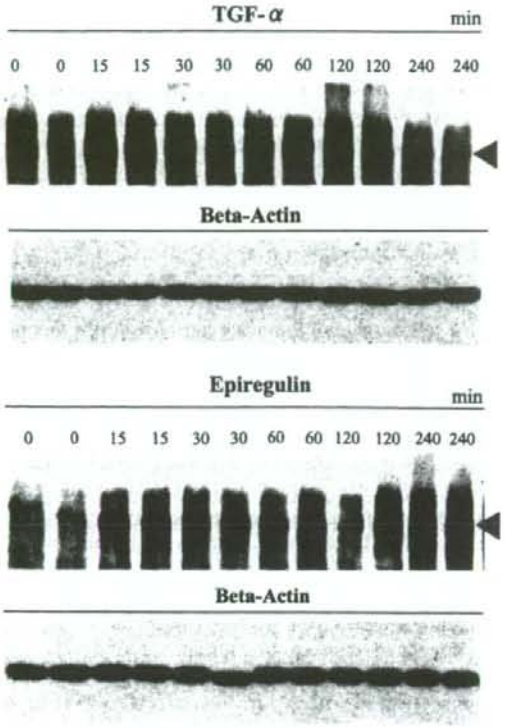

Fig. 1. Brain ErbB1 phosphorylation induced by subcutaneously administered TGF-α and epiregulin in mouse pups. Time courses of ErbB1 phosphorylation in the brain were determined after TGF-α or epiregulin injection at postnatal day (PND) 2. Solution of TGF-α or epiregulin (1.0 μg/g) was subcutaneously injected into neonatal C57BL/6N mice ($n = 12$ mice total for each group). The whole brain was dissected 0 (no injection), 15, 30, 60, 120, and 240 min after injection, and protein extracts were subjected to immunoblotting for anti-phospho ErbB1 antibody (two mice for each time point) and anti-β-actin antibody (control).

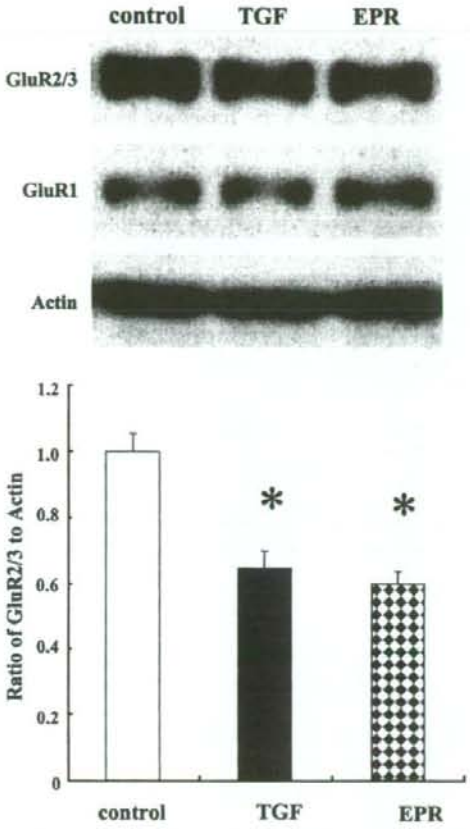

Fig. 2. Effects of repeated injections of TGF-α and epiregulin on AMPA receptor expression in the neocortex. Neonatal mice were daily treated with TGF-α, epiregulin (EPR), or saline from PND2 to PND10 and brain tissues were dissected out at PND11 when 24 h passed after the last injection was performed ($n = 5$ mice each). Protein was extracted from the neocortex and examined by immunoblotting with the antibodies directed against GluR1, GluR2/3, and β-actin. Immunoreactivity for GluR1 and GluR2/3 was normalized with that for β-actin in each lane. Mean protein level of control mice was set to 1.0. $^{*}p < 0.05$.

markedly stimulated eyelid opening (*two-tailed t-test*, $p < 0.001$) and tooth eruption (*two-tailed t-test*, $p < 0.001$). Similarly, epiregulin accelerated eyelid opening (*two-tailed t-test*, $p < 0.001$) and tooth eruption (*two-tailed t-test*, $p < 0.001$). The magnitude of the epiregulin-triggered acceleration for tooth eruption was less marked than that induced by TGF-α. The magnitudes of the TGF-α-triggered acceleration for physical development were, however, comparable to those induced by neonatal treatment with EGF (i.e., −5.2 day for eyelid, −2.1 day for tooth eruption) [17].

We also monitored the influence of TGF-α and epiregulin administration on body weight (Table 1). We compared mean body weight on PND10 when the last treatment was done as well as on PND60 when the mice grew to adults. In the postnatal stage, there was no significant alteration triggered by TGF-α or epiregulin treatment. In the adult stage, however, their effect on body weight emerged. A two-way ANOVA revealed a significant interaction between gender and TGF-α treatment ($F[1,21] = 8.527$, $p = 0.008$). Post hoc analysis found that neonatal treatment with TGF-α specifically elevated body weight of male mice. Epiregulin-treated male animals exhibited a similar trend in body weight that was not statistically significant ($F[1,25] = 2.638$, $p = 0.117$ for the interaction).

3.3. Influence of neonatal ErbB1 ligands on acoustic startle response and prepulse inhibition

Neonatal challenges of EGF are known to alter acoustic startle response and sensorimotor gating of rats and mice at their post-pubertal stages. Sensorimotor

Table 1
Effects of TGF-α and epiregulin challenges on physical development in mice

	Control	TGF-α
TGF-α		
Tooth eruption (PND)	10.80 ± 0.12	8.86 ± 0.09***
Eyelid opening (PND)	13.20 ± 0.12	9.85 ± 0.09***
Body weight (g)		
PND10	5.89 ± 0.09	5.59 ± 0.15
PND60 (male)	18.96 ± 0.75	21.47 ± 0.31*
PND60 (female)	18.13 ± 0.20	17.95 ± 0.42
	Control	Epiregulin
Epiregulin		
Tooth eruption (PND)	9.93 ± 0.16	8.80 ± 0.10***
Eyelid opening (PND)	13.0 ± 0.00	8.93 ± 0.15***
Body weight (g)		
PND10	5.86 ± 0.13	5.81 ± 0.11
PND60 (male)	20.91 ± 0.34	21.99 ± 0.28*
PND60 (female)	18.36 ± 0.41	18.08 ± 0.53

We monitored postnatal days (PND) when the tooth eruption and eyelid opening were first detected in mouse littermates receiving saline and TGF-α or those receiving saline and epiregulin. Body weight was also measured on PND10 and PND60 and compared between groups.
* $p < 0.05$.
*** $p < 0.001$.

gating is the neural process in which inhibitory neural pathways filter multiple stimuli to allow attention to be focused on one stimulus [19]. Sensorimotor gating can be assessed as PPI of startle, which is the modulation of the startle response, following a weak prepulse [20]. PPI magnitudes are decreased in several human neuropsychiatric disorders, including schizophrenia, obsessive–compulsive disorder, Huntington's disease, and Tourette's syndrome, suggesting that sensorimotor gating defects are a common neural dysfunction contributing to these psychiatric disorders [19,20].

We examined the effects of TGF-α and epiregulin on acoustic startle response in the presence or absence of four levels of prepulse tones at the young adult stage and separately analyzed data for TGF-α or epiregulin treatment as control littermates were set for each treatment (Fig. 3). Pulse-alone startle was significantly elevated at the higher ranges of acoustic stimuli. A multiple ANOVA for TGF-α employed the factors of treatment (2), gender (2), and sound intensity (7, repeated) and revealed significant main effects of TGF ($F[1,23] = 10.8$, $p = 0.003$) but not gender ($F[1,23] = 0.296$, $p = 0.592$) without an interaction between treatment and gender ($F[1,23] = 0.913$, $p = 0.349$) (Fig. 3A). Post hoc detected that TGF-α treatment increased pulse-alone startle at 110- and 120-dB tones. Similarly, neonatal epiregulin treatment elevated pulse-alone startle amplitudes ($F[1,25] = 6.56$, $p = 0.017$) without an interaction between treatment and gender ($F[1,25] = 1.302$, $p = 0.265$) (Fig. 3B). Comparisons with the basal startle levels for 75-dB tone revealed that treatment with TGF-α or epiregulin did not alter minimum thresholds of acoustic startle. These results suggest that the magnitudes or threshold levels of sound startle were not associated with the increases in startle amplitudes. Thus, neonatal treatment with TGF-α and epiregulin similarly enhances pulse-alone startle responses to 120-dB sound stimuli.

We simultaneously measured effects on PPI at the adult stage (Fig. 4). In TGF-α group, a multiple ANOVA with factors of gender (2), treatment (2), and prepulse intensity (4, repeated) yielded significant main effects for treatment ($F[1,23] = 10.79$, $p = 0.003$) and prepulse intensity ($F[1,23] = 69.2$, $p > 0.001$) but not gender ($F[1,23] = 0.549$, $p = 0.466$) (Fig. 4A). There was a significant interaction between treatment and gender ($F[1,23] = 6.29$, $p = 0.020$). There were significant main effects of TGF-α treatment at all prepulse levels ($F[1,23] = 4.46$–13.27, $p = 0.001$–0.046) and significant gender interactions only at 73- and 76-dB prepulses ($F[1,23] = 6.31$ and 5.28, $p = 0.019$ and 0.031, respectively). Post hoc revealed that PPI levels of saline-treated control males exhibited significantly higher PPI levels than control females at 73-dB prepulse ($p = 0.005$). In the epiregulin-treated mice, we observed the similar activity of epiregulin decreasing PPI levels. A multiple ANOVA revealed significant main effects for treatment ($F[1,25] = 6.56$, $p = 0.017$) and prepulse intensity ($F[1,25] = 135.1$, $p < 0.001$) but not gender ($F[1,25] = 1.22$, $p = 0.280$) without an interaction between treatment and gender ($F[1,25] = 1.302$, $p = 0.265$) (Fig. 4B). Post hoc detected the significant decrease in PPI levels at 76-dB and 82-dB prepulses ($p = 0.016$ and 0.008, respectively).

As neonatal treatments with TGF-α and epiregulin both simultaneously affected the pulse-alone startle and percentage PPI, interpreting the PPI decrease may be controversial and require evaluation [20]. To test the possibility that the increase in pulse-alone startle responses was responsible for the decrease in PPI, individual animal data for either TGF-α or epiregulin treatment were re-analyzed by the Pearson's correlation analysis (Fig. 5). When the percent PPI levels for 82-dB prepulse were plotted against the magnitude of the pulse-alone startle for each animal, there were no significant correlations between these values in TGF-α-treated group as well as in saline-treated controls ($p = 0.580$ for controls, $p = 0.911$ for TGF-α group) (Fig. 5A). The slope of the linear regression curve for the TGF-α-treated group was almost horizontal ($r = -0.033$). Both mathematical evaluations suggest that the TGF-α-triggered increase in pulse-alone startle is unlikely to contribute to the reduction PPI. Similar statistical results for the correlation between pulse-alone startle and %PPI in the TGF-α-treated group were obtained for the other prepulse levels ($p = 0.434$–0.682, $r = -0.121$–0.227).

We similarly applied the Pearson's correlation analysis to data of epiregulin-treated animals and their

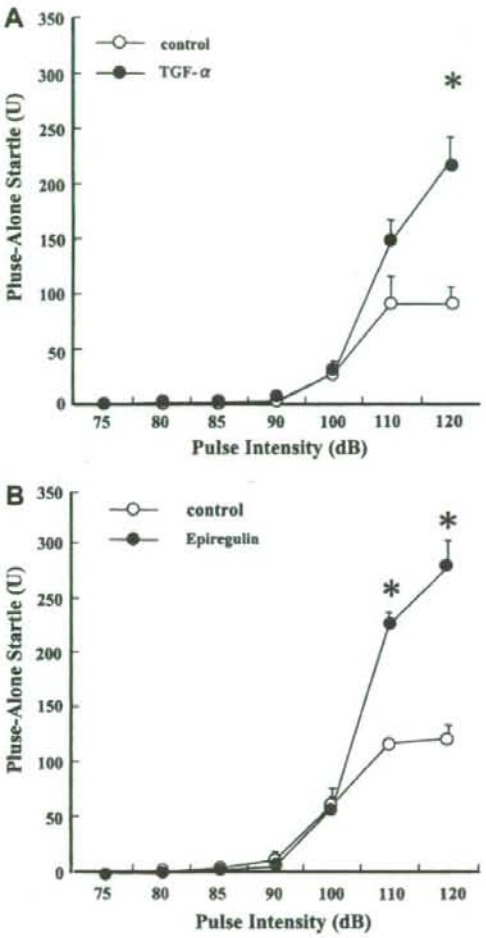

Fig. 3. Comparison of acoustic startle reactions. Neonatal mice were given TGF-α, epiregulin or saline ($n = 10$–17 for each group) during the early postnatal period as described above. At the adult stage (PND56-62), relative amplitudes of the startle response to 75-, 80-, 85-, 90-, 100-, 110-, and 120-dB white noise were measured and compared between littermates receiving TGF-α or saline (A) or receiving epiregulin or saline (B). Data represent mean ± SEM (arbitrary units). $^{*}p < 0.05$, a single startle level compared with saline-injected control littermates or $^{+}p < 0.05$ that with the background startle at 75-dB noise by Fisher PLSD. *Note:* The changes in startle amplitude were indistinguishable between males and females (data not shown).

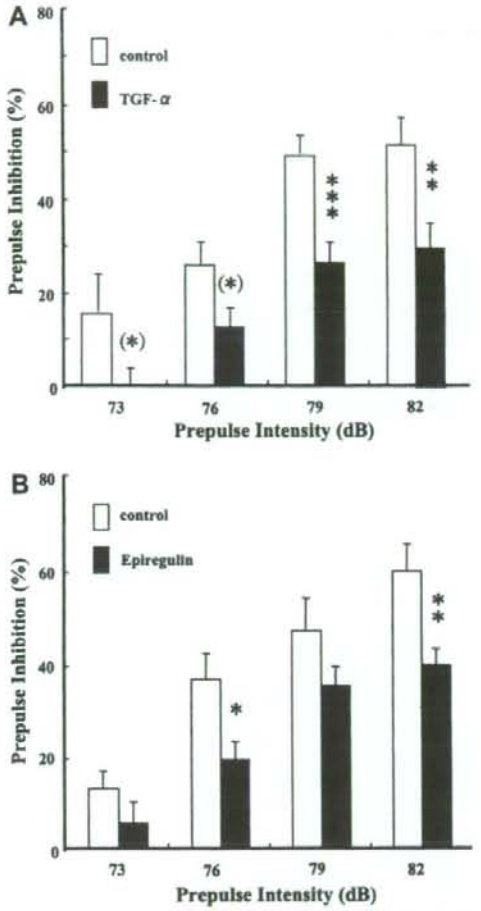

Fig. 4. Effects of neonatal administration of TGF-α and epiregulin on prepulse inhibition. Prepulse inhibition (PPI) was measured with 73-, 76-, 79-, and 82-dB prepulse stimuli in mice treated neonatally with TGF-α (A) or epiregulin (B) and compared with control littermates receiving saline ($n = 12$–17 for each group). Data represent mean ± SEM (% inhibition of main pulse responses). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, a single PPI level compared with saline-injected control littermates by Fisher LSD. (*) There were gender × treatment interactions at 73- and 76-dB prepulse levels; Male control mice exhibited significantly higher PPI levels; 65 ± 9% for male and 56 ± 8% for female at 73-dB prepulse, and 75 ± 9% for male and 65 ± 7% for female at 76-dB prepulse.

controls to estimate the potential effects of pulse-alone startle on %PPI. There was no significant correlation between pulse-alone startle and %PPI for all prepulses ($p = 0.130$–0.565 for controls and $p = 0.243$–0.755 for epiregulin group) (Fig. 5B). The regression curves for epiregulin-treated mice all displayed positive correlation coefficient ($r = 0.088$–0.321). Thus, we statistically conclude that epiregulin-triggered decreases in %PPI are not attributed to the increase in pulse-alone startle.

3.4. Influences of neonatal TGF-α and epiregulin treatments on exploratory motor behavior in adult mice

The effect of TGF-α and epiregulin on locomotor activity was assessed in a novel environment at young adult stage more than 6 weeks following their final injections (Fig. 6). In the experiment of TGF-α treatment, a multiple ANOVA with factors of treatment (2) × gender (2) × test duration (12, repeated) revealed no significant main effect of treatment ($F[1,23] = 0.00$, $p = 0.984$) and

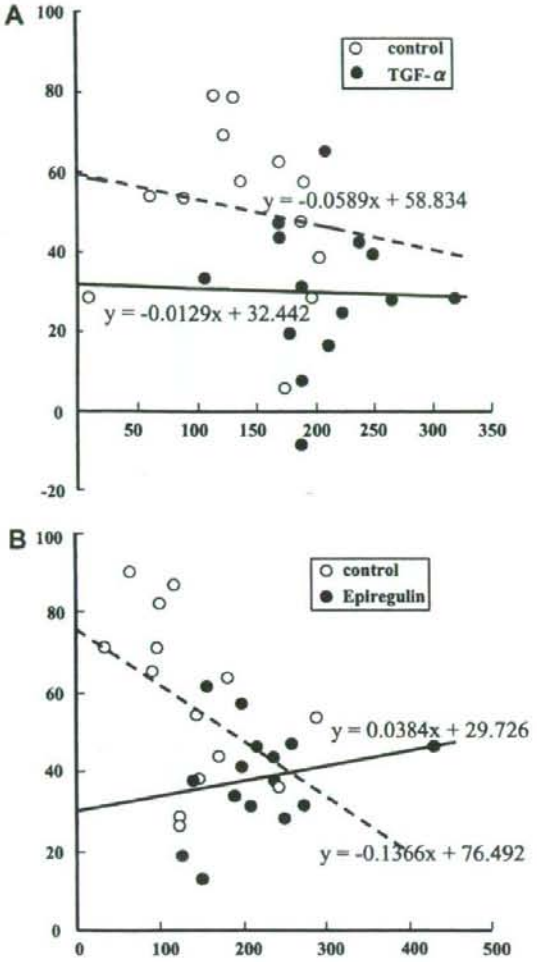

Fig. 5. Correlation analyses between %PPI and pulse-alone startle. (A) Percentage of PPI of the mice treated with vehicle (open circle, $n = 15$) and TGF-α (closed circle, $n = 15$) as neonates and plotted against pulse-alone startle. The data correspond to Fig. 4A. (B) Percentage of PPI of the mice treated with vehicle (open circle, $n = 15$) and epiregulin (closed circle, $n = 15$) was similarly plotted against the pulse-alone startle. The data correspond to Fig. 4B. A linear regression lines was estimated for each group and indicated with an equation. *Note:* There was no trend towards a decrease in the percentage of PPI versus absolute pulse-alone startle in either TGF-α-treated or epiregulin-treated animals.

gender ($F[1,23] = 1.630$, $p = 0.214$) without an interaction between treatment and gender ($F[1,23] = 1.167$, $p = 0.291$). Test duration had a significant overall effect ($F[1,23] = 268$, $P < 0.001$) without any factorial interactions. There was no significant effect of epiregulin on locomotor activity ($F[1,25] = 0.425$, $p = 0.521$) without an interaction between gender and treatment ($F[1,25] = 0.635$, $p = 0.433$). Thus, neonatal treatment with these ErbB1 ligands has no effect on exploratory locomotion at the adult stage.

3.5. Effects of ErbB1 ligands on contextual and auditory conditioning in mice

The effect of the ErbB1 ligands on learning was examined at the adult stage by measuring freezing behavior after training, in which an electric shock was coupled with a context plus a tone (Fig. 7). There was no significant difference in acoustic startle sensitivity with less than 100-dB sound stimuli (see Fig. 3). Freezing levels were compared separately for each treatment as well

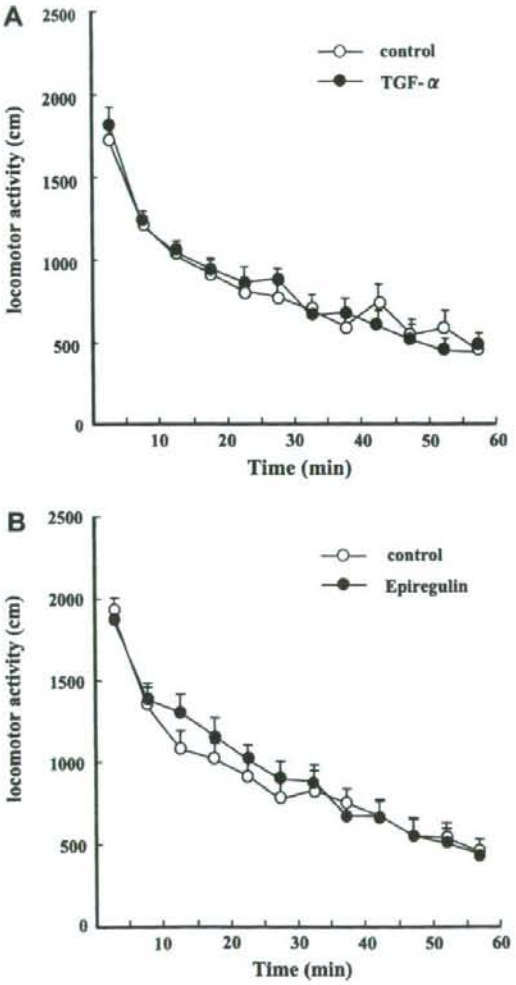

Fig. 6. Exploratory locomotor activity of mice treated with TGF-α and epiregulin. (A) We measured locomotor activity of mice (PND54-60) treated with TGF-α or saline ($n = 13$–17 each) as neonates in a novel environment. (B) Mouse littermates treated with epiregulin or saline ($n = 13$–17 each) were subjected to the same test at the adult stage. Horizontal activity in a novel open field was measured every 5 min and was plotted on the ordinate. Note: The changes in locomotor activity were indistinguishable between males and females (data not shown).

as in each testing session with ANOVA [treatment × gender × test duration (repeated, 6 points over the initial 3 min)]. In the TGF-α experiment, there were no significant main effects of TGF-α treatment on freezing time coupled with the context cue ($F[1,23] = 0.000$, $p = 0.996$) and the tone cue ($F[1,23] = 0.018$, $p = 0.896$) (Fig. 7A). Neonatal treatment with epiregulin did not alter learning performance in either paradigm ($F[1,27] = 0.351$, $p = 0.559$ for context and $F[1,25] = 0.070$, $p = 0.793$ for tone) (Fig. 7B). In both experiments, there were no significant differences in freezing rate during conditioning between ErbB1 ligand-treated mice and saline-treated controls, suggesting no influence of neonatal TGF-α or epiregulin treatment on shock sensitivity ($F[1,23] = 1.166$, $p = 0.291$ for TGF-α and $F[1,25] = 3.118$, $p = 0.090$ for epiregulin). There was no gender effect or no gender × treatment interaction in the above learning performance, either (data not shown). Thus, neonatal exposure to neither TGF-α or epiregulin influences shock-triggered fear-learning ability.

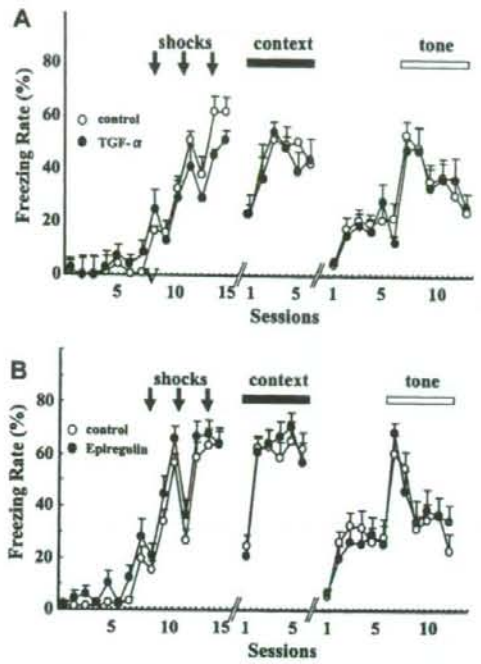

Fig. 7. Tone-dependent and context-dependent learning in TGF-α- and epiregulin-treated mice. (A) TGF-α- and saline-treated mice ($n = 12$–16 each) were subjected to shock-paired contextual conditioning with a tone cue at PND63-70 and, after 1 d, their learning performance was measured with the contextual cue and the tone cue. Mean percentage (±SEM) of freezing time of TGF-α-treated mice (closed circle) was scored every 30 s during conditioning as well as during testing periods (3 min) and compared with that of control littermates receiving saline as neonates (open circle). (B) Learning performance of epiregulin-treated mice was determined with the same protocol. Arrows mark the timing of electric shock given. Open and closed bars indicate the timing of the context cue given and that of the tone cue given during test sessions, respectively. *Note:* Freezing time was indistinguishable between males and females in all test sessions (data not shown).

4. Discussion

Our previous studies demonstrated that peripherally-administered inflammatory cytokines influence development of immature neurons in postnatal brain and later produce a variety of behavioral and cognitive abnormalities [13,24,25]. Adult endophenotypes of behavioral abnormalities vary depending upon cytokine species administered as well as on animal strains [17,26]. In the present study, however, we learned that the distinct cytokines, acting on the same ErbB1 receptor, can produce the same behavioral phenotypes. Neonatal administration of TGF-α and epiregulin both increased acoustic startle response and decreased PPI levels at the adult stage. These ErbB1 ligands both failed to affect exploratory movement and fear-learning ability, however. Thus, the observed behavioral impairments induced by these ErbB1 ligands considerably resembled those triggered by neonatal challenge of EGF [17], although there were differences in the magnitudes of brain ErbB1 phosphorylation as well as in those of acceleration of physical development among the ErbB1 ligands. As the transgenic mice carrying the EGF expression vector also a similar behavioral abnormality (HN unpublished data), it is unlikely that a minor compound(s) in the samples of TGF-α and epiregulin might produce the behavioral deficits.

Considering the fact that serum, milk and amniotic fluids contain effective concentrations of various ErbB1 ligands such as EGF, TGF-α, amphiregulin and betacellulin [6–9,27], the present study demonstrates that all ErbB1 ligands in the peripheral tissues or fluid of neonates may have common latent effects on brain development and synergistically influence adult behavioral traits, irrespective ErbB1 ligand spices. In the case of the rat model established with interleukin-1 injection as neonates, there is a clear time window for both cytokine penetration through the blood–brain barrier and later behavioral consequences [28]. In this context, we assume that the permeability of TGF-α and epiregulin to the blood–brain barrier of mouse neonates similarly has crucial impact on later behavioral traits.

Transgenic mice carrying TGF-α transgene and natural TGF-α hypomorphic mutant mice (*wa-1*) have been analyzed previously [29–32]. The male transgenic mice are reported to be aggressive in the resident-intruder test and depressive in the enforced swim test [30]. The behavioral deficits are ameliorated by administration of serotonin uptake inhibitors [29]. Similarly, male mice carrying *wa-1* allele exhibited impaired auditory and contextual fear learning with a decrease in hippocampal

volume [31]. Again, there were significant gender differences in monoamine metabolism of *wa-1* mutants [32]. In contrast to these reports, our procedure of the neonatal TGF-α administration did not produce gender-specific alterations in behaviors, although there was a significant gender × treatment interaction in adult body weight. In addition, there was no significant effect of TGF-α and epiregulin on fear-learning performance in the present study. As we found the modest change of EGF-challenged rats in monoamine metabolism but not that in brain structures [13,18], the peripheral challenge of TGF-α and epiregulin in the neonatal period of time may also produce an influence on brain monoamine metabolism at the adult stage, leading to the behavioral changes.

Neonatal treatment with TGF-α and epiregulin both facilitated eyelid opening and tooth eruption, as that with EGF does [10]. There was difference in the magnitude of acceleration in physical development, however. Epiregulin exhibited a lower potency of accelerating tooth eruption than TGF-α at the same dose. In agreement with this finding, the receptor phosphorylation following epiregulin injection was also delayed and less pronounced, compared with animals receiving TGF-α injection. TGF-α triggered phosphorylation of ErbB1 in the brain peaked and disappeared within 240 min after injection while EGF administration to rat neonates results in sustained phosphorylation of ErbB1 [13]. In spite of the differences in receptor activation and physical development, the subchronic influences of TGF-α and epiregulin on AMPA receptor expression as well as on adult behaviors were almost comparable. The discrepancy in peripheral vs. central or acute vs. chronic effects of these ErbB1 ligands awaits future investigations.

Maternal infection during pregnancy, birth complications and maternal care of neonates are all suggested to influence later psychobehavioral traits or a risk for schizophrenia or other neurodevelopmental disorders [32–34]. ErbB1 ligands appear to be potential candidate molecules that mediate these pathological processes following infection, inflammation, and psychological stimuli. Endogenous expression of ErbB1 receptor ligands (EGF, TGFα, HB-EGF, and amphiregulin) is induced by neuronal activity, inflammation, and injury [35–38]. Rearing conditions also affect the production of TGFα in the neonatal brain [39]. As patients with schizophrenia exhibited abnormal expression of EGF in the brain, we previously focused on EGF among many ErbB1 ligands and characterized acute and intensively investigated chronic influences of EGF on behaviors. The present results, however, indicate that EGF is not the sole player leading to the behavioral deficits involving ErbB1 signaling, although there is no direct evidence that abnormal expression of any ErbB1 ligand in human subjects results in schizophrenia or other neurodevelopmental disorders.

These ErbB1 ligands exert common biological activities on various types of neurons. EGF, HB-EGF, and TGF-α have strong trophic activity on midbrain dopaminergic neurons [15,40–42]. These ErbB1 ligands also have a de-differentiation activity on GABAergic neurons [22] and an inhibitory action on growing excitatory synapses [43]. Our recent studies indicate that ErbB1 stimulation with EGF or TGF-α has an activity to down-regulate AMPA receptor expression [22,23]. The finding contrasts with the other report showing that the inhibition of neuregulin-1/ErbB4 signaling similarly down-regulates AMPA receptors [44]. Although it remains unknown which biological activity of ErbB1 signals play a main pathological role in the behavioral impairments, these activities appear to all match the schizophrenia hypotheses for the GABAergic, dopaminergic, and glutamatergic systems proposed previously. We hope that investigations on ErbB1-triggered behavioral impairments will help elucidating psychiatric pathology and etiology, leading to novel therapeutic inventions.

Acknowledgements

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Brief report

IQ decline and memory impairment in Japanese patients with chronic schizophrenia

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Abstract

The extent of IQ decline due to the development of illness in patients with chronic schizophrenia and the degree of memory impairment relative to such IQ decline still remain unclear. Our results suggest that schizophrenia patients experience marked IQ decline due to the development of illness and their wide-ranging memory impairments are even more severe than the IQ decline.

Keywords: Schizophrenia; IQ; Memory

1. Introduction

Cognitive impairment is a core feature of schizophrenia, with a great impact on patients' daily lives. Those therapies that have the potential to improve cognitive deficits of patients with schizophrenia, including cognitive remediation therapy (Medalia et al., 1998; Wykes et al., 2003), as well as the favorable effects of atypical antipsychotic drugs on cognition (Bilder et al., 2002; Harvey et al., 2006; Keefe et al., 2006), have been attracting increasing attention from researchers and clinicians. From this viewpoint, the precise delineation of cognitive impairments in schizophrenia patients is essential.

Intellectual deficits in patients with chronic schizophrenia have been reliably identified (Heinrichs and Zakzanis, 1998; Dickinson et al., 2004) with some ongoing debate as to "whether it is possible to be schizophrenic yet neuropsychologically normal" (Palmer et al., 1997; Kremen et al., 2000; Wilk et al., 2005); however, the extent of IQ decline caused by the development of schizophrenia remains unclear because the premorbid IQ scores of persons who later develop schizophrenia are lower than

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those of their peers (Fuller et al., 2002; Reichenberg et al., 2005). Impairments in memory, working memory, and attention in patients with schizophrenia are well documented (Aleman et al., 1999; Silver et al., 2003; Hori et al., 2006), but the relationship of these cognitive deficits to the possible decline in IQ has not been established. Here we assessed cognitive functions including intellectual and wide-ranging memory functioning in patients with chronic schizophrenia in relation to age- and premorbid IQ-matched healthy controls.

2. Materials and methods

Eighty-two patients who met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia participated in this study. All patients were receiving antipsychotic drugs at the National Center of Neurology and Psychiatry (NCNP), Musashi Hospital and were clinically stable at the time of the neuropsychological tests. Eighty-two age- and premorbid IQ-matched healthy volunteers were recruited from hospital staff and their associates and also from the community. Healthy participants were interviewed by a research psychiatrist using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Sheehan et al., 1998) to confirm the absence of any psychiatric illnesses. A portion of the subjects were from our previous sample (Hori et al., 2006). Written informed consent was obtained from all subjects prior to their inclusion in the study. The study was approved by the ethics committee of the NCNP.

Premorbid IQ was estimated with the Japanese Adult Reading Test (JART, Matsuoka et al., 2002; 2006), a Japanese version of the National Adult Reading Test (NART, Nelson and Wilson, 1991). This test is considered to provide an estimate of premorbid IQ in schizophrenia patients (Uetsuki et al., 2006), which is consistent with the original NART (Crawford et al., 1992; O'Carroll et al., 1992). In this test, subjects were required to read out 100 idioms of Han-Chinese characters (Japanese kanji characters). JART-estimated premorbid IQ was calculated for each subject according to previous reports (Matsuoka et al., 2002, 2006). The full version of the Wechsler Memory Scale-Revised (WMS-R, Wechsler, 1987; Sugishita, 2001) was administered to all participants. Outcome measures of the WMS-R were verbal memory, visual memory, delayed recall, auditory attention, visual attention, verbal working memory, and visual working memory. To precisely assess subjects' current intellectual function, a full version of the Wechsler Adult Intelligence Scale-Revised (WAIS-R, Wechsler, 1981; Shinagawa et al., 1990) was administered, yielding age-corrected indices of verbal, performance, and full-scale IQs.

Schizophrenic symptoms were assessed by an experienced research psychiatrist in 46 of the 82 patients using the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). Daily doses of antipsychotics and anticholinergic antiparkinsonian drugs were converted to chlorpromazine equivalents (CPZeq) and biperiden equivalents (BPDeq), respectively, using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999; Minzenberg et al., 2004).

Results are reported as mean ± standard deviation (S.D.). Demographic characteristics and cognitive test results were compared between groups. We used *t*-test or analysis of variance (ANOVA) to compare mean scores and the χ^2 tests to compare categorical variables. Analysis of covariance (ANCOVA) was used to compare means between groups, controlling for confounding variables. Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

Male/female ratios of patients and controls were 48/34 and 25/57, respectively, indicating that the patient group had a greater representation of males ($\chi^2(1)=13.06$, $P<0.001$). The mean ages of the patients and controls were 44.3±13.8 and 44.2±14.9, respectively ($t=0.05$, $df=162$, $P=0.96$). The mean years of education of the patients and controls were 13.4±2.5 and 14.1±2.2, respectively ($t=1.88$, $df=162$, $P=0.06$). The JART-predicted premorbid IQ scores of patients and controls were 102.2±11.6 and 102.3±7.4, respectively ($t=0.46$, $df=137.8$, $P=0.96$). Of the 82 patients, 56 were outpatients and 26 were inpatients. The mean age of illness onset was 24.7±8.8. Illness duration was 19.6±13.7 years, demonstrating that our patients were in the chronic phase of schizophrenia. CPZeq and BPDeq were 781.7±710.1 and 2.2±2.0, respectively. PANSS positive, negative, and total scores were 13.9±6.7, 19.1±7.1, and 62.1±17.9, respectively.

Verbal, performance, and full-scale IQs of patients with schizophrenia and healthy controls are presented in Supplementary Table 1. ANOVA showed that these three IQ indices in patients were significantly lower than those in controls (all $P<0.001$). The VIQ/PIQ ratios of patients and controls were 1.08±0.18 and 0.95±0.11, respectively ($F=22.5$, $df=1,160$, $P<0.001$, by ANCOVA with gender as a covariate). Scores of 13 subscales of the WMS-R in patients and controls are also shown in Supplementary Table 1. Patients performed significantly

more poorly than controls on all these cognitive domains (all $P<0.001$), except for auditory attention ($P=0.15$). Fig. 1(a) shows mean scores of the patients and controls on JART-estimated IQ, WAIS-R full-scale IQ, and the main three memory indices of the WMS-R. Dips of current IQ and all memory domains in patients are apparent, although the two groups are matched for the JART-estimated premorbid IQ.

To control for the current IQ and gender effects on these test results, ANCOVA was used with full-scale IQ and gender as covariates. It revealed that patients performed significantly more poorly than controls on verbal memory, visual memory, delayed recall, visual attention, and verbal working memory, even after controlling for full-scale IQ and gender (Supplementary Table 1). To confirm these results, additional comparisons were made between patients whose current IQ scores were within normal limit (IQ-WNL patients, defined as WAIS-R full-scale IQ ≥ equal to or greater than 85; $n=46$) and total controls ($n=82$). Fig. 1(b) summarizes the results, showing that there was no difference in current IQ between IQ-WNL patients (mean IQ: 98.85±8.55) and controls (mean IQ: 101.95±11.30), while these patients still showed significantly lower scores on all three memory indices compared with controls. On the other hand, the JART-estimated premorbid IQ of IQ-WNL patients was significantly higher than that of controls.

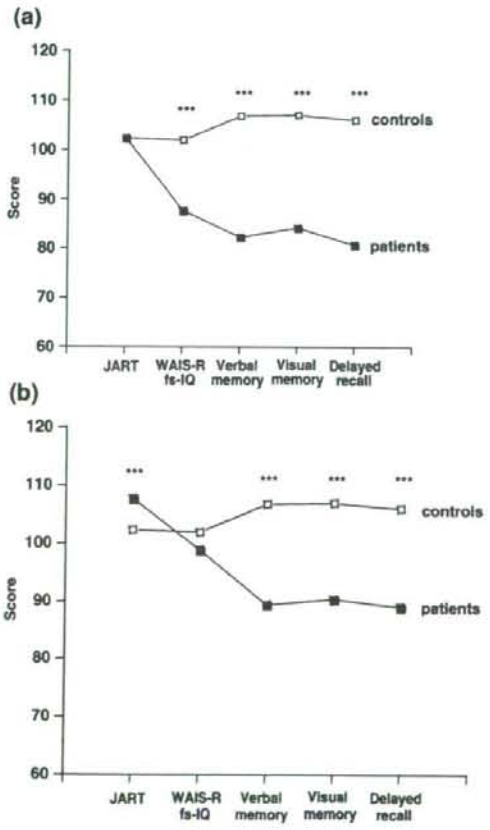

Fig. 1. Mean scores of patients and controls on JART IQ, WAIS-R full-scale IQ, Verbal memory, Visual memory, and Delayed recall indices (WMS-R). (a) total patients ($n=82$) vs. total controls ($n=82$) and (b) IQ-WNL patients (defined as WAIS-R full-scale IQ ≥ 85, $n=46$) vs. total controls ($n=82$).***$P<0.001$.

4. Discussion

In the present study we examined intellectual and memory functions in patients with chronic schizophrenia relative to age- and premorbid IQ-matched healthy controls. Our results confirmed that patients with chronic schizophrenia have wide-ranging cognitive impairments, consistent with the literature on schizophrenia.

The relationship of the development of schizophrenia to declining IQ scores has been confounded by findings that premorbid intelligence itself is likely to be lower in persons who later develop schizophrenia than in their peers (Fuller et al., 2002; Reichenberg et al., 2005). To address this issue, we employed a premorbid IQ-matched case-control sample. Although the cross-sectional nature of the present study does not allow any definite conclusions to be drawn concerning the time when the IQ decline actually occurred (i.e., during the prodromal stage, immediately after illness onset, or during the chronic course of illness), the observed differences in current IQs between patients and controls provide evidence for marked IQ decline due to the development of schizophrenia. Means of estimated premorbid IQ and current full-scale IQ in patients were 102.20 and 87.68, respectively, suggesting an approximate 1 S.D. decline in IQ score related to the development of illness. On the other hand, the subgroup of patients whose current IQ was within normal limits (and thus similar to that of controls) showed significantly higher premorbid IQ as estimated by the JART than controls (Fig. 1(b)), which favors the view that even neuropsychologically normal patients with chronic schizophrenia have compromised cognitive functioning relative to their presumed premorbid level of intellectual function (Kremen et al., 2000). Furthermore, in the present study performance IQ of the patients was more severely impaired than verbal IQ, congruent with prior reports (Heinrichs and Zakzanis, 1998).

Pervasive memory impairment in patients with schizophrenia relative to premorbid IQ-matched controls was

found, and most deficits remained significant even after current IQ was controlled for, supporting that memory impairment is a core feature of schizophrenia (Saykin et al., 1991; Heinrichs and Zakzanis, 1998; Aleman et al., 1999). The marked impairment in verbal memory is consistent with numerous studies (e.g., Saykin et al., 1991; Heinrichs and Zakzanis, 1998). Although visual memory deficits in schizophrenia have attracted less attention from researchers than verbal memory, several studies have reported substantial impairment of visual memory (Saykin et al., 1991; Aleman et al., 1999), consistent with the present study. The pronounced impairment in delayed recall observed here is also in line with prior reports (Aleman et al., 1999; Dickinson et al., 2004). Deficits of verbal and spatial working memory in schizophrenia tapped by the Wechsler digit span backward and spatial span backward subtests, respectively, are fairly consistent findings (Conklin et al., 2000; Silver et al., 2003; Dickinson et al., 2004), which were replicated in the current study. Previous studies have reported that the performance on the forward digit span task of schizophrenia patients is significantly poorer than that of healthy people, indicating impaired attentional function in schizophrenia (Conklin et al., 2000; Silver et al., 2003). The findings of the present study, by contrast, suggest that auditory attention as measured by the forward digit span subtest is preserved in schizophrenia. The discrepant findings regarding auditory attention in the present study relative to previous ones might be due in part to the distinct matching status between patients and controls regarding education and premorbid IQ.

In conclusion, our results suggest that patients with chronic schizophrenia have substantially lower intellectual function relative to their presumed premorbid level and that their memory impairment is even more severe than the IQ decline. To definitively delineate the lifetime course of cognitive decline in schizophrenia, longitudinal studies that range from childhood to the chronic phase are needed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2007.11.002.

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