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Platelet-Derived Growth Factor B-Chain Homodimer Suppressing a Convulsion of Epilepsy Model Mouse El

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El mouse is a mutant which has epileptic convulsions after tossing-up stimulations and has a hippocampal dysfunction. Platelet-derived growth factor B-chain homodimer has been reported to be a trophic factor of hippocampal neurons. We found that a recombinant PDGF-BB suppressed the convulsions of El mice in a dose-dependent manner. Furthermore, thrombin-treated mice manifested no convulsions, but thrombin receptor activating peptide-treated ones had convulsions. These findings suggest that an abnormality in PDGF-BB release may make El mice susceptible to tonic-clonic convulsions.

The mutant inbred strain El mouse was established from the ddY mouse which has no epileptogenicity. The El mice manifest epileptiform seizures after several weeks of successive tossing-up stimulations about 10 cm height once a week from the age of 4 weeks [1]. The typical seizure is composed of prodromal, tonic-clonic convulsive and postictal stage [2]. Metabolic and structural abnormalities of hippocampal neurons in El mice were observed in a number of neurochemical studies. Those studies suggested that the seizure of El mice has relations to dopamine [3], 5-hydroxytryptamine [4] or opioids [5] and that the uptake of γ-aminobutyric acid (GABA) [6] and norepinephrine [3] were decreased in the hippocampus. Therefore, El mice are considered as an animal model of human temporal epilepsy.

It has been reported that PDGF-BB prolongs the life span of the hippocampal neurons and promotes the formation of their axons and dendrites [8]. Therefore, we hypothesized that PDGF-BB might act on hippocampal neurons to affect convulsions in El mice.

Herein, the role of PDGF-BB in convulsions was investigated in El mice. First, we examined the effect of exogenous PDGF-BB on the tonic-clonic convulsion of El mice. Next, as thrombin induces platelets and vascular endothelial cells to secrete PDGFs [9,10], we also investigated an anti-convulsive effect of endogenous PDGF on the convulsion.

METHODS

Animals. El mice were obtained from the Shizuoka Laboratory Animal Cooperative Association, Inc. (Hamamatsu, Japan), and were reared under the specific pathogen-free conditions with a 12H : 12H light dark cycle. Male El mice were given successive 40 tossing-up stimulations about 10 cm height once a week from the age of 4 weeks until the age of 13 weeks. All El mice manifested a typical seizure within 40 tossing-up stimulations.

Examination of anti-convulsive effect. Human thrombin (Enzyme Research Lab., USA), thrombin receptor activating peptides (TRAP) (Cosmo Bio Co., Japan), a recombinant human PDGF-BB and PDGF-AA (Bachem California Co., USA) were separately dissolved in 0.9% NaCl (physiological saline). Hundred μl of these samples and physiological saline were injected intraperitoneally to groups of 7 El mice. The stimulation test was performed by a unit of 40 tossing-up stimulations about 10 cm height and an interval of 5 min. Maximum stimulation was composed of 5 times of 40 tossing-up stimulations and 4 intervals of 5 min between stimulations. The susceptibility of an individual mouse to convulsions was expressed as

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Abbreviations: GABA, γ-aminobutyric acid; PDGF-AA, platelet-derived growth factor A chain homodimer; PDGF-BB, platelet-derived growth factor B chain homodimer; TRAP, thrombin receptor activating peptides.

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a number of tossing-up stimulation times to manifest tonic-clonic convulsions. The statistical evaluation of the data was performed by the use of Wilcoxon's U-test.

RESULTS

PDGF-BB Suppressed the Convulsion in El Mice

The degree of lowering susceptibility to convulsions is expressed as numbers of tossing-up stimulation to induce convulsions. In a normal mouse, more than 200 stimulations do not induce convulsions. On the other hand, 40 or less stimulations can induce convulsions in El mice.

In order to examine directly the effect of PDGF on convulsions, we injected various doses of a recombinant human PDGF-BB and PDGF-AA intraperitoneally into El mice and induced convulsions 24 hr after the injection (Fig. 1). PDGF-BB suppressed convulsions in a dose-dependent manner. However, 1000 ng of PDGF-AA had no significant effect on sensitivity to convulsions as compared to that of saline-treated control. Next, we investigated how long this anti-convulsive effect continued (Fig. 2). The anti-convulsive effect was not recognized 2 hr after the injection of PDGF-BB. When 10 ng of PDGF-BB was injected, suppressive effect to convulsions was maximum in 24 hr and decreased rapidly. When 100 ng of PDGF-BB was injected, the effect continued until 72 hr and then decreased in 168 hr. In mice treated with 1000 ng of PDGF-BB, convulsions were completely suppressed in 24 hr and 72 hr and almost blocked in 168 hr. The strength and duration of anti-convulsive effect were in a dose-dependent manner. These findings revealed that PDGF-BB completely suppressed the convulsions in El mice.

Anti-convulsive Effect of Thrombin and Thrombin Receptor Activating Peptide (TRAP)

Next we investigated whether the synthesis of PDGF-BB was defective in El mice. Thrombin induced platelets and vascular endothelial cells to secrete PDGFs[9]. TRAP binds to the thrombin receptor and increases the synthesis of PDGF but does not induce secretion of PDGFs[10]. Human

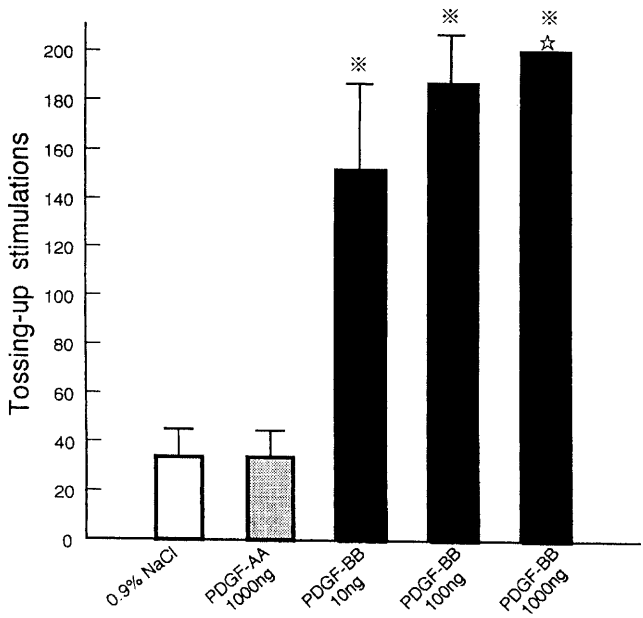

FIG. 1. Anti-convulsive effect of human platelet-derived growth factor A chain homodimer (PDGF-AA) and B chain homodimer (PDGF-BB). PDGF-AA or PDGF-BB was dissolved in 100 μl of 0.9% NaCl and injected intraperitoneally into 7 El mice. The mouse was given tossing-up stimulations (10 cm height) 24 hr after the injection. Mean and S.D. were calculated in a group of 7 El mice. *$p < 0.01$ as compared with controls (Wilcoxon's U-test). ☆indicates that the mice had no convulsion after 200 stimulations were given.

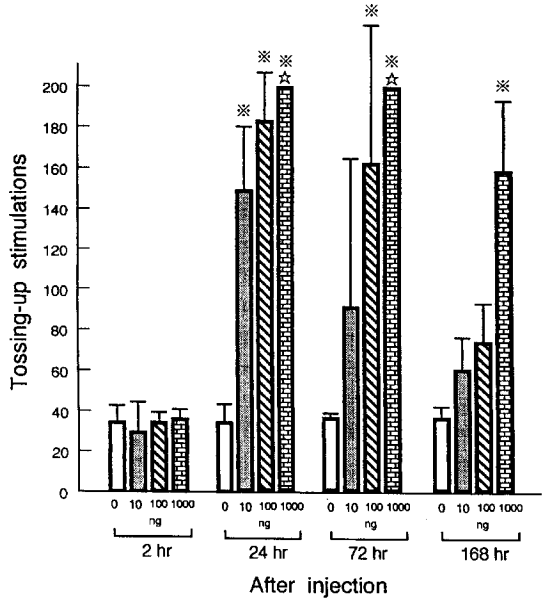

FIG. 2. Duration of anti-convulsive effect of human PDGF-BB. Various amounts of PDGF-BB were dissolved in 100 μl of 0.9% NaCl and injected intraperitoneally into 7 El mice. The mouse was given tossing-up stimulations (10 cm height) at 2, 24, 72, and 168 hr after the injection. Mean and S.D. were calculated in a group of 7 El mice. *p < 0.01 as compared with controls (Wilcoxon's U-test). ☆indicates that the mice had no convulsion after 200 stimulations were given.

thrombin or TRAP was injected intraperitoneally to 7 El mice and the anti-convulsive effect was examined 24 hr after the injection (Fig. 3). Forty ng of thrombin decreased sensitivity to convulsions a little. Large doses (400 ng and 4000 ng) of thrombin completely suppressed convulsions. However, large dose (4000 ng) of TRAP has no effects. These findings suggest that PDGF-BB was synthesized, stored normally and released into blood by thrombin in El mice.

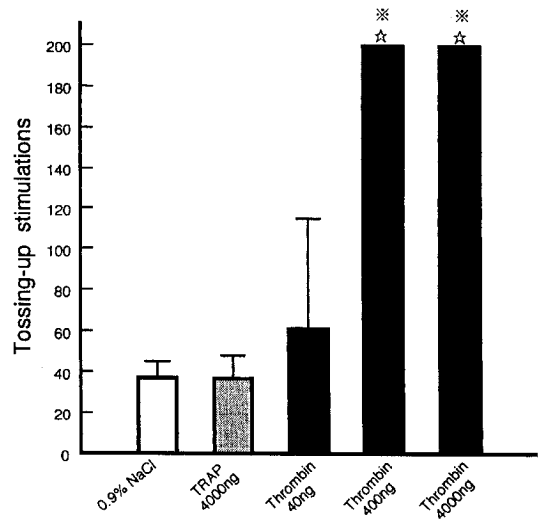

FIG. 3. Anti-convulsive effect of human thrombin and thrombin receptor activating peptides (TRAP). Thrombin and TRAP were dissolved in 100 μl of 0.9% NaCl and injected intraperitoneally into 7 El mice. The mouse was given tossing-up stimulations (10 cm height) 24 hr after injection. Mean and S.D. were calculated in a group of 7 El mice. *p < 0.01 as compared with controls (Wilcoxon's U-test). ☆The same as in Fig. 2.

DISCUSSION

Temporal-lobe epilepsy is considered to be caused by abnormal functions of hippocampal neurons. Abnormalities in neurotransmitters [3–6] in El mice have been reported by a number of investigators. However, the exact mechanisms regulating convulsions in these mice are not known.

In this study we investigated the effect of PDGF-BB on convulsions in El mice and found that PDGF-BB has a close relation to susceptibility to tonic-clonic convulsions in El mice. Furthermore, when we injected thrombin to release endogenous PDGFs, the convulsion was completely blocked. Our results suggest that the release of PDGF-BB by unidentified stimulus, but not synthesis of PDGF-BB, might be defective in El mice.

The anti-convulsive effect of PDGF-BB was observed 24 hr after the injection but not after 2 hr. This result indicates that PDGF-BB may modify functions of hippocampal neurons rather than act on them as a neurotransmitter. This hypothesis is supported by several lines of evidence. First, the hippocampal neurons have a PDGF receptor-β and PDGF-BB prolongs the life span of the hippocampal neurons and promotes the formation of their axons and dendrites [8]. Interestingly, During et al. [11] has reported that glutamate-induced GABA release in decreased is human temporal-lobe epilepsy. It is also known that PDGF-BB has a trophic activity to GABAergic neurons of rat cerebellum *in vitro* [8] and that glutamate-induced GABA release *in vivo* is mediated by GABA transporter in rats[11]. Therefore, it seems possible that PDGF-BB may increase the GABA transporter which in turn uptakes GABA in the hippocampal neurons of El mice and decreases the excitability to generate convulsions. Second, Matsumoto et al.[12] has reported that the absence of inositol triphosphate receptor in brain induces epileptic seizure. Increased inositol triphosphoric acid formation by 1-amino-1,3-cyclopentane-trans-dicarboxylic acid was observed in hippocampus of ddY mice, but not of El mice [13]. As PDGFs increase inositol triphosphoric acid of various cells [14,15], PDGF-BB may increase inositol triphosphoric acid in hippocampal neurons of El mice and suppress the convulsion.

Although the mechanism that PDGF-BB completely suppresses convulsions in El mice, an animal model of human temporal-lobe epilepsy, is not currently clarified, our finding is an important milestone for basic and clinical research for the epilepsy. Further investigation of the mechanism of the anti-convulsive action will develop a new therapeutic drug for epilepsy.

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