Increase in drug-induced seizure susceptibility of transgenic mice overexpressing GABA transporter-1

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ABSTRACT

AIM: The changes of seizure susceptibility of transgenic mice overexpressing GABA transporter-1 (GAT-1) were studied to clarify the possible role of GABAergic transmission in epileptogenesis. **METHODS:** Seizures were induced by intraperitoneal administration of pentylenetetrazol (PTZ), picrotoxin (PIC), or kainic acid (KA) respectively. The anticonvulsant effect of ethyl nipecotate was tested by its intraperitoneal injection 15 min before the administration of the epileptogenic agents. **RESULTS:** The percentages of occurrence of clonic seizures induced by PTZ 45 mg/kg, PIC 2.5 mg/kg, or KA 20 mg/kg in GAT-1 transgenic mice were 88.9 %, 100 %, and 83.3 % respectively, whereas those in control C57BL/6J mice were 42.9 %, 57.1 %, and 33.3 %. The percentages of occurrence of tonic seizures induced by PTZ 45 mg/kg, PIC 2.5 mg/kg, or KA 20 mg/kg in transgenic mice were 88.9 %, 100 %, and 83.3 % respectively, and whereas those in control mice were 28.6 %, 42.9 %, and 16.7 %. The latencies of both clonic and tonic seizures onset in transgenic mice were markedly shortened compared with those in control animals. The results indicated that GAT-1 transgenic mice showed increased susceptibility to seizures induced by the anti-GABAergic convulsive drugs (PTZ, PIC), as well as glutamic receptor agonist (KA). Ethyl nipecotate, inhibitor of GAT-1, inhibited PTZ-induced seizures in both GAT-1 transgenic and C57BL/6J mice. The incidence of seizures was decreased after the application of ethyl nipecotate, and the latencies to the onset of clonic or tonic seizures were also prolonged. **CONCLUSION:** The increase in seizure susceptibility of transgenic mice over-expressing GAT-1 is an evidence for involvement of GABAergic transmission in epileptogenesis, and this transgenic mouse might be a useful animal model for study on the role of GABAergic transmission in epileptogenesis.

INTRODUCTION

Impaired GABAergic system has been generally considered as a principal factor in the pathogenesis of epilepsy. Extracellular GABA concentration was regulated mostly by GAT-1 in presynaptic terminals and in glial cells[1]. During *et al* reported that the function of GAT-1 was impaired in epileptogenic hippocampi of patients with temporal lobe epilepsy[2]. Moreover, the repetitive seizures induced by pentylenetetrazol (PTZ) were inhibited by injection of NNC711, a known inhibitor of GAT-1, in rats[3]. Pharmacological blockade of GABA transporter by tiagabine (TGB) or by nipecotic acid inhibited audiogenic seizure and reduced neuronal firing in the inferior colliculus of genetically epilepsy-

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prone rats[4,5]. Thus, it was evident that GAT-1 should be involved in the pathologenesis of certain types of epilepsy. In the present work, the changes in seizure susceptibility of transgenic mice overexpressing GAT-1, the predominant GABA transporter in the rodent brain, were examined in order to obtain further evidence for the involvement of GABA-transmission in epileptogenesis.

It is well known that PTZ and picrotoxin (PIC) are considered to act at the picrotoxin receptor of $GABA_A$ receptor complex[6], and kainic acid (KA) is a potent and selective agonist of central excitatory amino acids[7]. These agents were used to induce seizures, and the role of GABA-transsmission in epileplogenesis was discussed in relation to excitatory glutamine transmission.

MATERIALS AND METHODS

Animals A full length cDNA coding for murine GAT-1 (mGAT-1) which was screened from the λ phage murine brain cDNA library was cloned into the *Eco*R I and *Apa* I site of pcDNA3, under the control of human cytomegalovirus (CMV) promoter/enhancer. This recombinant plasmid, linearized with *Nru* I and *Tth*111 I and subsequently gel-purified, was microinjected into the pronuclei of fertilized eggs of (C57BL/6J) F1 hybrid mice (Jackson Laboratory, USA). Polymerase chain reaction (PCR) and Southern-blot analysis were carried out with tail DNA to verify the integration of variable copy numbers of transgene into the genomes of founder mice and their progenies. Semi-quantitative reverse transcription-PCR and Northern-blot analysis of whole RNA samples, extracted from a variety of tissues, were used to characterize the expression pattern of the transgene. The details of these procedures have been described previously[8].

Male and female GAT-1 transgenic mice (n=56) and age-matched nontransgenic C57BL/6J mice (n=91) used in present work were (75±15) d of age and were maintained on a 12 h light/12 h dark cycle and permitted free access to food and water.

Drugs and seizure susceptibility test PTZ (Serva), PIC (Fluka AG), KA (Sigma), and ethyl nipecotate (Acros organics) dissolved in physiological saline were administered intraperitoneally in a volume of 1-2 mL for one animal. Ethyl nipecotate was injected 15 min before the administration of PTZ to test its inhibitory effect on seizures. All solutions were freshly prepared daily.

Prior to measurement of seizure susceptibility, all animals were placed in an air-conditioned testing room for at least 1 h. Mice were kept individually in the cage (450 mm×350 mm×300 mm) for observation of seizure activity. Observation period was lasted 120 min after drug administration. The onset of clonic or tonic seizure was defined as the first occurrence of clonus or tonic seizure respectively[9]. Clonic seizure was considered as having occurred when mouse showed rhythmic jerks or wild running. Tonic seizure was characterized by continuous tension or contraction of muscles. Each mouse was used only once in experiment.

Double-blind testing was employed: the experimenters conducting the behavioral experiment were blinded to the genotypes of the mice, and the genotype analysts performing the PCR analysis did not know the results of seizure susceptibility tests.

Statistical analysis Statistical examination of differences in the occurrence of seizures was carried out by SAS 6.12 (SAS Institute Inc, USA) chi-square test. The results for latencies to the onset of clonic or tonic seizures were expressed as mean±SD and statistical differences were evaluated by SAS 6.12 analysis of variance (ANOVA). P<0.05 indicated that the difference was statistically significant.

RESULTS

Changes in susceptibility of GAT-1 transgenic mice to drug-induced seizures The occurrence of seizures in both GAT-1 transgenic and age-matched C57BL/6J control mice increased with increasing doses of all three convulsive drugs and the percentages of occurrence of seizures in transgenic mice were much higher than those in control mice. Differences between seizure susceptibility of GAT-1 transgenic mice and that of control group were significant (P<0.05 or P<0.01). The results were shown in Fig 1 and Fig 2. PTZ 35 mg/kg induced clonic seizures in 75.0 % (6 out of 8) of transgenic mice and in 10.5 % (2 out of 19) of control mice respectively, whereas the tonic seizures were induced in 62.5 % (5 out of 8) of transgenic mice, and none of control animals exhibited tonic seizure. The differences between two groups were significant (P<0.01). PTZ 45 mg/kg induced clonic seizures in 88.9 % (8 out of 9) of transgenic mice, but only in 42.9 % (6 out of 14) of control animals the seizures were induced. The difference between them was statistically significant (P<0.01). At the same time the

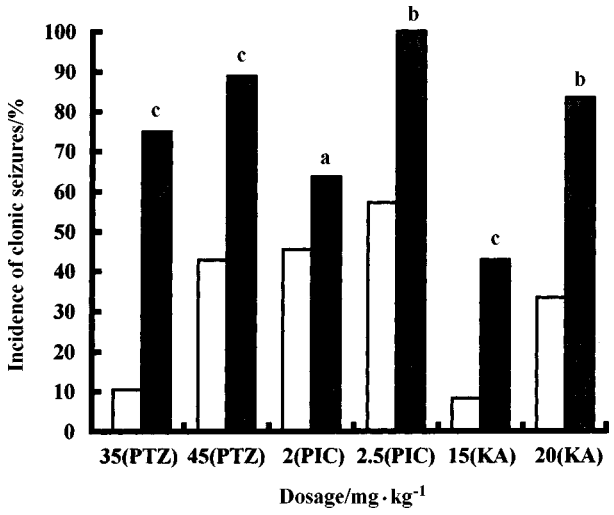

Fig 1. Percentages of mice exhibiting clonic seizures following administration of three convulsive drugs. Open bars indicated C57BL/6J control mice and filled bars indicated GAT-1 transgenic mice. PTZ: pentylenetetrazol; PIC: picrotoxin; KA: kainic acid. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* C57BL/6J control mice.

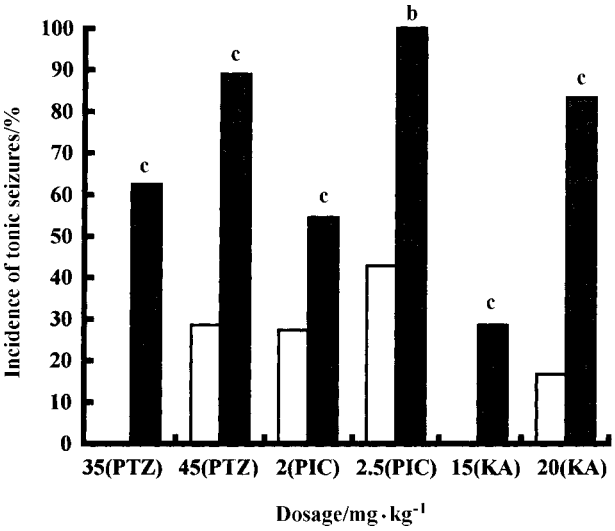

Fig 2. Percentages of mice exhibiting tonic seizures following administration of three convulsive drugs. Open bars indicated C57BL/6J control mice and filled bars indicated GAT-1 transgenic mice. PTZ: pentylenetetrazol; PIC: picrotoxin; KA: kainic acid. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* C57BL/6J control mice.

tonic seizures were induced by PTZ 45 mg/kg in 88.9 % (8 out of 9) of transgenic and in 28.6 % (4 out of 14) of control mice. The difference between two groups was significant ($P<0.01$). After PIC 2 mg/kg administration, 63.6 % (7 out of 11) of GAT-1 transgenic and 45.5 % (5 out of 11) of control mice exhibited clonic seizures. As for PIC-induced tonic seizures, the occurrence percentages in transgenic and control mice were 54.6 % (6 out of 11) and 27.3 % (3 out of 11) respectively. The similar results were obtained with higher dose 2.5 mg/kg of PIC administration. The clonic as well as the tonic seizures were induced by PIC 2.5 mg/kg in all transgenic mice used (5 out of 5). However, in 57.1 % (4 out of 7) of control animals the clonic seizures were induced, and 42.9 % (3 out of 7) of control mice exhibited tonic seizures. The difference between them was statistically significant ($P<0.05$). After KA 15 mg/kg administration 42.9 % (3 out of 7) of GAT-1 transgenic mice exhibited clonic seizures and only 8.3 % (1 out of 12) of control animals did. When the dose was increased to 20 mg/kg, the percentages of occurrence of clonic seizures were 83.3 % (5 out of 6) in transgenic and 33.3 % (4 out of 12) in control mice respectively. The difference between them was statistically significant ($P<0.05$). KA 15 mg/kg induced tonic seizures in 28.6 % (2 out of 7) of transgenic mice, but none of control animals exhibited tonic seizures. After KA 20 mg/kg injection, 83.3 % (5 out of 6) of transgenic and 16.7 % (2 out of 12) of control mice exhibited tonic seizures. The difference between two groups was significant ($P<0.01$).

The latencies of both clonic and tonic seizures in transgenic mice were significantly shorter than those in control mice. Two-way ANOVA analysis revealed significant differences between latencies to the onset of clonic seizures in GAT-1 transgenic and control mice for PTZ ($P<0.01$), PIC ($P<0.05$), and KA ($P<0.05$)-induced clonic seizures, as well as those for tonic seizures (PTZ: $P<0.05$; PIC: $P<0.05$; KA: $P<0.05$, Tab 1).

Inhibitory effect of ethyl nipecotate on drug-induced seizures After injection of ethyl nipecotate 50 mg/kg 15 min before PTZ 45 mg/kg injection, only one (1 out of 8) GAT-1 transgenic mouse exhibited tonic seizures, and none of C57/BL6J mice (0 out of 8) exhibited seizures. The inhibitory effect of ethyl nipecotate on the induction of clonic seizures was also investigated. There were only two mice exhibited clonic seizures either in GAT-1 transgenic group (n=8) or in C57BL/6J group (n=8). In the control experiments after administration of physiological saline 15 min before injection of PTZ the clonic seizures were induced in eight mice on transgenic group (n=10) as well as in C57BL/6J group (n=16). In physiological saline control experiments, the latencies to the onset of clonic seizures were (4.2±2.5) min and (7±4) min in GAT-1

Tab 1. Latencies to the onset of clonic or tonic seizures induced by pentylenetetrazol, picrotoxin, and kainic acid in GAT-1 transgenic and control (C57BL/6J) mice. Numbers in parenthesis represented animals showing clonic or tonic seizures out of total tested animals. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* C57BL/6J control mice.

Epileptogenic agents/ mg·kg^{-1}	Seizures	Latency of onset /min	
		Control mice	GAT-1 transgenic mice
Pentylenetetrazol			
35	Clonic	32.0±1.0 (2/19)	6.2±2.8^{c} (6/8)
	Tonic	0 (0/19)	6.3±1.2^{b} (5/8)
45	Clonic	7±4 (6/14)	4.2±2.5^{c} (8/9)
	Tonic	8.5±2.6 (4/14)	4.9±1.8^{b} (8/9)
Picrotoxin			
2	Clonic	41±11 (5/11)	24±12^{b} (7/11)
	Tonic	42±4 (3/11)	30±13^{b} (6/11)
2.5	Clonic	33±10 (4/7)	11±6^{b} (5/5)
	Tonic	31±5 (3/7)	15±8^{b} (5/5)
Kainic acid			
15	Clonic	51.5±0.0 (1/12)	42±4^{b} (3/7)
	Tonic	0 (0/12)	38.5±2.1^{b} (2/7)
20	Clonic	44±5 (4/12)	24±8^{b}(5/6)
	Tonic	44±4 (2/12)	27±9^{b} (5/6)

transgenic and C57BL/6J mice respectively. After the administration of ethyl nipecotate, the latencies to onset of clonic seizures were (30.0±2.0) min and (38.4±0.4) min in C57BL/6J and GAT-1 transgenic groups respectively. Our results showed that ethyl nipecotate significantly alleviated PTZ-induced seizures in both GAT-1 transgenic and C57BL/6J mice, compared with those injected with physiological saline. Their differences were statistically significant ($P<0.01$).

DISCUSSION

Our experiment clearly showed that the increase in seizure susceptibility of transgenic mice overexpressing GAT-1 was not only to GABA-related drugs (PTZ and PIC), but also to glutamic receptor agonist (KA). The seizure susceptibility of transgenic mice was significantly higher than that in their age-matched controls. These results suggested that GAT-1 might play an important role in epileptogenesis.

Transporters were generally thought to indirectly modulate neurotransmission by maintaining low concentrations of extracellular transmitters, and as a secondary function they prevented extracellular transmitter levels from reaching toxic levels. Such increased seizure susceptibility in GAT-1 transgenic mice might be related to the alteration in the balance of excitatory and inhibitory synaptic interactions in these mice. There was compelling evidence that epilepsy was associated with changes in the balance of glutamatergic and GABAergic neurotransmission[10-13]. More recently it was suggested that changes in GABA and glutamate transporters took an important role in epileptogenesis and seizure propagation. The alterations in extracellular GABA and glutamate concentrations might be related to transporter functions in epilepsy patients[14,15], and GABA transporter proteins were reported to be up-regulated in animals undergoing iron-induced epileptogenesis[16]. In the present study, the balance of excitatory and inhibitory synaptic transmission should be affected in GAT-1 transgenic mice due to the over-expression of GAT-1, and consequently their susceptibility to seizures induced by both anti-GABAergic convulsive drugs (PTZ and PIC) and excitatory receptor agonist (KA) was increased. In our previous study[17], it was found that $GABA_A$ receptor and glutamate transporters were up-regulated in GAT-1 transgenic mice, which might reflect a compensatory or corrective change against the overexpression of GAT-1, and contributed to maintain the balance of excitatory and inhibitory synaptic transmission in these mice. So the over-expression of GAT-1 in such mice might decrease GABA levels by facilitating re-uptake of GABA into the GABA pool and decreased extracellular GABA levels would result in the increased excitation in drug-induced seizures. Recent findings, however, suggested that transporters maybe had direct effects on postsynaptic receptor functions[18]. That was another possible cause responsible for the increased seizure susceptibility in GAT-1 transgenic mice. Further studies will be needed to elucidate the underlying mechanisms by which GAT-1 altered the seizure susceptibility.

The increase in seizure susceptibility of transgenic mice over-expressing GAT-1 and the inhibition of drug-induced seizures by ethyl nipecotate suggested that the alteration in GAT-1 might be an etiological factor for certain type of epilepsy. Given those drugs with an action similar to that of ethyl nipecotate, *ie*, drugs that increased the extracellular GABA levels by blocking GABA transporters, might be effective in seizure control.

Support for this view was provided by the data on TGB. Being directly against GABA transporters, TGB was known as a potent anticonvulsant agent against DMCM-induced clonic convulsions (mice), PTZ-induced tonic convulsions (mice and rats), sound-induced convulsions in DBA/2 mice and genetically epilepsy-prone rats, and electrically-induced convulsions in kindled rats. TGB was currently in phase II/III clinical trials for treatment of epilepsy in Europe and the United States[19]. The overall findings in GAT-1 transgenic mice and anticonvulsant profiles of GAT-1 inhibitors suggested potential utility of GAT-1 inhibitors in the treatment of epilepsy.

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