

## Glutamate and GABA concentrations in the cerebellum of novel ataxic mutant *Pogo* mice

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The *Pogo* mouse is an autosomal recessive ataxic mutant that arose spontaneously in the inbred *KJR/MSKist* strain derived originally from Korean wild mice. The ataxic phenotype is characterized by difficulty in maintaining posture and side to side stability, faulty coordination between limbs and trunk, and the consequent inability to walk straight. In the present study, the cerebellar concentrations of glutamate and GABA were analyzed, since glutamate is a most prevalent excitatory neurotransmitter whereas  $\gamma$ -aminobutyric acid (GABA) is one of the most abundant inhibitory neurotransmitters, which may be the main neurotransmitters related with the ataxia and epilepsy. The concentration of glutamate of cerebellum decreased significantly in ataxic mutant *Pogo* mouse compared to those of control mouse. However, GABA concentration was not decrease. These results suggested that the decrease in glutamate concentration may contribute to ataxia in mutant *Pogo* mouse.

**Key words:** *Pogo*, glutamate, GABA, cerebellum

### Introduction

The *Pogo* mouse is an autosomal recessive ataxic mutant that arose spontaneously in the inbred *KJR/MSKist* strain derived originally from Korean wild mice. The ataxic phenotype is characterized by difficulty in maintaining posture and side to side stability, faulty coordination between limbs and trunk, and the consequent inability to

walk straight [16,18]. The *Pogo* mutation is inherited as a trait on chromosome 8 as well as the tottering, leaner, and rolling mutations.

Glutamate is the most prevalent excitatory neurotransmitter [6], whereas  $\gamma$ -aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter [20]. Glutamate is the main excitatory neurotransmitter in the brain [10] and all glutamate is formed from glucose within the central nervous system because glutamate does not readily cross the blood-brain barrier [11,15,21,25]. Glutamate is synthesized from 2-oxoglutarate by transamination either with alanine, aspartate or one of the branched chain amino acids leucine, isoleucine and valine, and can also be formed from glutamine by phosphate-activated glutaminase [28]. Glutamate is accumulated into vesicles to a high concentration and released to the synapses by calcium-dependent exocytosis upon the arrival of an action potential. As a high extracellular concentration of glutamate is also neurotoxic, high-affinity glutamate transporters are essential for terminating synaptic transmission and for maintaining a low extracellular glutamate concentration.

GABA is the primary inhibitory neurotransmitter known to counterbalance the action of the excitatory neurotransmitter glutamate. The importance of GABA as an inhibitory neurotransmitter in the mammalian cerebellum is well documented [23,27]. The excitatory granule cells, by far the most numerous neuronal type in the cerebellum [9], receive input from GABAergic cells; thus it has been suggested that the majority of GABA receptors are located on granule cells [24]. GABA is thought to be released from the interneurons by feedforward inhibition from the granule cells or by feedback inhibition from the pyramidal cells controlled by

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glutamatergic nerve endings [12]. The present study examined that the concentrations of glutamate and GABA in the cerebellum of *Pogo* mouse.

## Materials and Methods

### Animals

Mice were generated from a breeding colony of *Pogo* mice developed from original breeding pairs obtained from Korea Research Institute Bioscience and Biotechnology (KRIBB). 30-day-old ataxic *Pogo* (*pogo/pogo*) and normal wild mice (+/+, control) were used in all experiments. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

### Immunohistochemistry

All mice were deeply anaesthetized with sodium pentobarbital (60 mg/kg body weight) and transcardially perfused with 0.9% NaCl in 0.1 M phosphate buffer saline (PBS, pH 7.4) followed by 150 ml of 4% paraformaldehyde in 0.1 M PBS (pH 7.4). These brains were removed from the skull and placed immediately in the same fixative at 4°C for 24 hours. The post-fixed brains were transferred to 0.1 M PBS, and after the brains were cryoprotected in 10%, 20% and 30% sucrose in 0.1 M PBS and cryostat sectioned in the frontal plane 20 µm thickness. After several rinses in 0.1 M PBS (pH 7.4) the sections were quenched for 10 min in 1% H<sub>2</sub>O<sub>2</sub>, and rinsed in 0.1 M Tris phosphate-buffered saline (TPBS; 8.5 mM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 3 mM KH<sub>2</sub>PO<sub>4</sub>, 125 mM NaCl, 30 mM Tris-HCl, 0.03 mM NaN<sub>3</sub>, pH 7.7). Sections were incubated overnight at room temperature in rabbit polyclonal anti-calbindin-D (anti-CaBP, Sigma Inc., St. Louis MO). They were then washed three times for 5 min in 0.1 M TPBS, and incubated in 1:100 peroxidase-conjugated anti-rabbit IgG (Dakopatts Inc., Mississauga, Canada) for 2 hours at room temperature. After three additional rinses in TPBS, antibody-binding sites were revealed by a 15 min incubation in 0.2% diaminobenzidine in TPBS. Sections were then dehydrated through graded

**Table 1.** Condition of HPLC for the determination of brain glutamate and GABA concentration in mice

Parameter	Conditions
Column	RP-C <sub>18</sub> (150 × 4.0 mm I.D., 10 µm)
Flow rate	0.6 ml/min
Mobile phase	10 mM potassium acetate buffer (pH 6.5)-methanol
Gradient	Methanol 20-70%/40 min
Attenuation	10
Detector	Fluorescence detector (λ <sub>ex</sub> : 340 nm, λ <sub>em</sub> : 450 nm)

alcohols and mounted in DPX (BDH Chemicals Inc., Toronto, Canada).

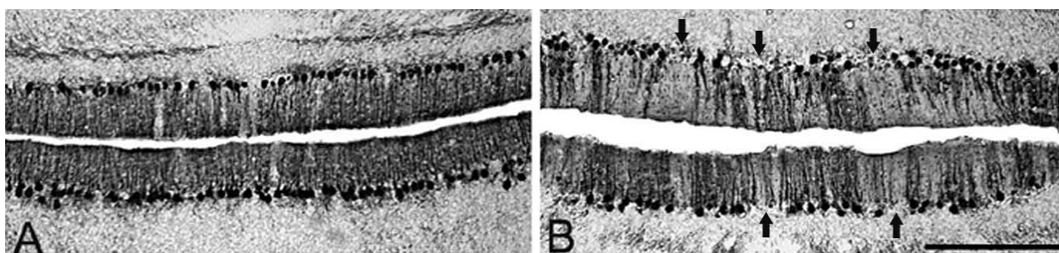
### Determination of glutamate/ GABA levels

Concentrations of glutamate and GABA in the cerebellums were measured using a modified method of Allen *et al.* [1]. Tissues were homogenized in 0.3 M triethanolamine buffer, pH 6.8, containing of 1 mM aminoethylisothiuronium bromide and 2 mM pyridoxal 5'-phosphate, then centrifuged (Hanil Supra 22K, ROK) at 15,000 g for 20 mins. Postmitochondrial fraction from each extract was resuspended in 20 mM potassium phosphate buffer, deproteinized, and then centrifuged. Supernatants were filtered by membrane filter (0.2 µm: 13 mm), and then o-phthalaldehyde derivatives were used for the detection of fluorescence in the HPLC measurement (fluorescence detector, SHIMADZU, Japan, Table 1). The amounts of glutamate and GABA in cerebellums were represented as nmole per mg protein.

## Results

### A. Immunohistochemistry

Calbindin is expressed in the cerebellum exclusively by Purkinje cells [8,22]. Anti-CaBP immunohistochemistry deposited peroxidase reaction product throughout all Purkinje cells, including the somata, dendrites, dendritic spines and axons, in both normal wild type and *pogo/pogo*

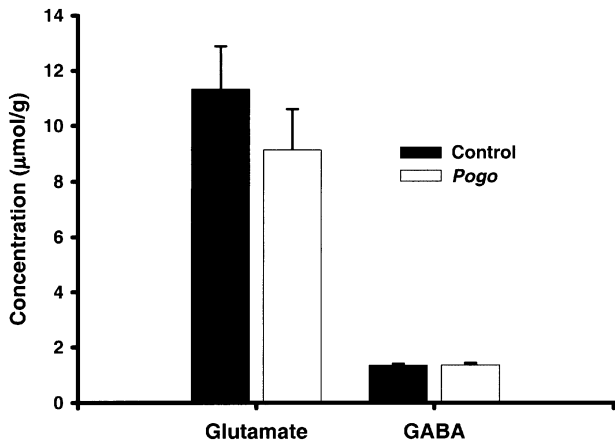


**Fig. 1.** Anti-calbindin immunoreaction were showed in frontal sections through the vermis of the cerebellum of a +/+ normal wild type mouse [control] (A) and ataxic *Pogo* mouse [*pogo/pogo* homozygote] (B) in lobule VIII and IX. The loss of Purkinje cells (arrow) in ataxic *Pogo* mouse is seen when compared with corresponding lobule of the +/+ normal wild type mouse. Scale bar = 100 µm.

**Table 2.** Concentrations of glutamate and GABA in cerebellums

	Glutamate ( $\mu\text{mol/g}$ )	GABA ( $\mu\text{mol/g}$ )
Control	$11.327 \pm 1.561$	$1.353 \pm 0.055$
<i>Pogo</i>	$9.147 \pm 1.457^*$	$1.360 \pm 0.074$

\*Data are represented as Mean  $\pm$  S.D. of 6-9 animals. \* $p < 0.05$ : Significantly different from control



**Fig. 2.** Concentrations of glutamate and GABA in the cerebellums of control and ataxic *Pogo* (*pogo/pogo*) mice.

mutant mice (Fig. 1). The Purkinje cells appeared normal in *pogo/pogo* mutant mice with respect to their size and arrangement as a monolayer in the Purkinje cell layer (Fig. 1). Purkinje cell ectopia was rare. Individual Purkinje cells in the vermis of *pogo/pogo* mutant mice are grossly normal with parasagittally oriented dendritic arbors extending to the surface of the molecular layer (Fig 1A). However, in *pogo/pogo* mutant mice there was a loss of Purkinje cells throughout the cerebellar vermis (Fig. 1B). The loss of somata and dendrites of Purkinje cells was clearly demonstrated by using anti-CaBP immunostaining (Fig. 1B).

### B. Concentration of glutamate and GABA of cerebellum

As illustrated in Table 2 and Fig. 2 the concentration of glutamate of cerebellum decreased significantly in ataxic mutant *Pogo/Pogo* mouse compared to those of control mouse. But GABA concentration was not decrease.

## Discussion

In our present experiment, the concentration of glutamate decreased in *pogo/pogo* mouse. There have been several reports of glutamate and GABA concentration in weaver, Purkinje cell degeneration (PCD), leaner and E1 mouse cerebellum. In weaver mice cerebellum, neuronal loss occurs during postnatal development and leads to a partial Purkinje cell degeneration, an almost complete loss

of granule cells and their parallel fibers and, in consequence, to the formation of 'heterologous' mossy fiber contacts on Purkinje cells and a persistent multi-innervation of Purkinje cells by olivary climbing fibers [7,26]. The phase relationships to sinusoidal vestibular stimulation of floccular Purkinje cells are greatly distorted and irregular under these conditions making a precise time matching of convergent input onto target neurons in the vestibular nuclei highly uncertain [13,14]. In addition, sprouting and enlargement of GABAergic synaptic boutons in the dorsal part of the lateral vestibular nuclei was observed recently in this mutant [2,3]. In Purkinje cell degeneration mutants, where cell loss affects the mature cerebellum, a clear increase in somatal parvalbumin-immunoreactivity in the vestibular nuclei and deep cerebellar nuclei suggests an enhanced activity of mainly inhibitory neurons. However, GABAergic reinnervation was not found [2,4,5]. In leaner, reinnervative reactions of both Purkinje cell GABAergic and extracerebellar GABAergic sources, that would substitute for the lost Purkinje cell-input, are not indicated by the present findings using GABA-immunohistochemistry. GABAergic innervation density is diminished to one-half in the dorsal part of the lateral vestibular nuclei of leaner, which is only slightly higher than in Purkinje cell degeneration mutants [2]. This reduction corresponds well with the massive Purkinje cell loss in the anterior lobe and shows that, in contrast to weaver, GABAergic reinnervation does not occur under these conditions. In addition, GABAergic terminals in leaner are reduced in size to such a degree, comparatively only with that found after experimental removal of the cerebellum or in Purkinje cell degeneration mutants [2].

Then, there is a report of the glutamate concentration in E1 mouse. The E1 mouse is a genetically susceptible model of complex-partial epilepsy with secondary generalization of seizures. This model shows elevated GABA (40-50%) and lowered glutamine and glutamate (30%) in its most epileptic state E1 (+) compared with control or E1 (-) mice (i.e. same genetic type but not multiply stimulated to become responded to handling by having seizures). However, there was a great increase in glutamate level during the pre-convulsive state, and the seizures themselves were blocked by AP5 given intraventricularly 30 mins before seizure induction, in which GABA levels increased transiently immediately after seizures [19]. Thus both glutamatergic and GABAergic systems appear to be central to the mechanisms generating seizures in the E1 mouse [17].

In this study, we have provided that the concentration of glutamate in *pogo/pogo* mouse cerebellum decreased compared to control mouse. However, GABA concentration was not changed.

These results suggested that the reduction of glutamate

concentration may related with disarrangement in synapse and contribute to motor ataxia in *Pogo* mouse.

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