

Spontaneous Electrical Activity in Cerebellar Purkinje Neurons of Postnatal Rats

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Although cerebellar Purkinje cells display spontaneous electrical activity *in vivo* and in slice experiments, the mechanism of the spontaneous activity generation has not been clearly understood. The aim of this study was to investigate whether cerebellar Purkinje cells of postnatal rats generate spontaneous electrical activity without synaptic inputs. Dissociated cerebellar Purkinje cells were used for reducing synaptic inputs in the present study. Cerebellar Purkinje cells with dendrites were dissociated from postnatal rats using enzymatic treatment followed by mechanical trituration. Spontaneous electrical activities were recorded from dissociated cells without any stimulus using whole-cell patch clamp configuration. Two types, spontaneously firing or quiescent, of dissociated Purkinje cells were observed in postnatal rats. Both types of cells were identified as Purkinje cells using immunocytochemical staining technique with anti-calbindin after recording. Spontaneously active cells displayed two patterns of firing, repetitive and burst firings. Two thirds of dissociated Purkinje cells displayed repetitive firing and the rest of them did burst firing under same recording condition. Repetitive firing activities were maintained even after further isolation using either physical or pharmacological techniques. Neither high magnesium solution nor excitatory synaptic blockers, AP-5 and DNQX, block the spontaneous activity. These results demonstrate that spontaneous electrical activity of isolated cerebellar Purkinje cells in postnatal rats is generated by intrinsic membrane properties rather than synaptic inputs.

Key Words: Cerebellar Purkinje neurons, Spontaneous activity, Repetitive firing, Burst firing, Calbindin antibody, Cell isolation

INTRODUCTION

Spontaneous electrical activity of neurons has been investigated to better understand motor behaviors of invertebrates and vertebrates, and certain neurological disorders of vertebrates (Delcomyn, 1980; Llinas, 1988). Further it has been recently known that spontaneous activity plays an important role in synaptic connections and neuronal circuit development. For example, spontaneous activity was involved in the establishments of connections at many different stages

throughout the visual pathway (Shatz, 1990; Goodman & Shatz, 1993). Spontaneous activity of one or a few trigger neurons in the neocortex activates the rest of the cells resulting in the formation of a neuronal domain (Yuste et al, 1995). In the cerebellum electrical activity plays an important role in the timing properties of motor execution that characterizes the cerebellar control of motor coordination (Welsh et al, 1995).

Since cerebellar Purkinje cells receive numerous inputs through climbing and parallel fibers and are the sole source of output from the cerebellar cortex, their activity is very important in various cerebellar functions. *In vivo* experiments demonstrated that electrical activity of Purkinje cells was closely related

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to various movements such as limbs (Thach, 1967), head and smooth-pursuit eye movements (Lisberger & Fuchs, 1978). Spontaneous electrical activity of Purkinje cells can be generated by either intrinsic membrane properties or synaptic inputs. There is indirect evidence indicating that Purkinje cells display endogenous electrical activity *in vivo* and *in vitro* experiments. Purkinje cells displayed spontaneous activity either before synapses formed or after the excitatory pathway were eliminated *in vivo* (Woodward et al, 1974; Crepel et al, 1981). Half of cultured Purkinje cells examined in high Mg^{2+} saline also displayed spontaneous activity *in vitro* (Gahwiler et al, 1973). Bursting electrical activity recorded from adult Purkinje neurons in cerebellar slices persisted in the presence of TTX (Llinas & Sugimori, 1980). However, no direct evidence that Purkinje cells generates electrical activity by intrinsic membrane properties exists. Moreover, Hockberger et al (1989) did not observe spontaneous electrical activity in acutely isolated Purkinje cells of postnatal rats. Thus the source of spontaneous electrical activity and ionic mechanisms underlying the spontaneous activity in Purkinje cells is still unclear.

I examined whether spontaneous activity of Purkinje cells was generated by intrinsic membrane properties using acutely isolated Purkinje cells. Results in the present study demonstrate that spontaneous repetitive firing activity of dissociated Purkinje cells with dendrites is generated by endogenous membrane properties.

METHODS

Animals

Postnatal Sprague-Dawley rats were used. Birth-date of offspring was determined within 12 hrs after delivery, and this period together with the next 12 hrs was designated as postnatal day 1 (P1). P7 to P14 rats were used in most experiment. There were a few experiments using three week-old rats. Young rats were decapitated using a guillotine or a scissors. The heads were placed in an antibiotic-isotonic buffered saline at 4°C, and the cerebella were separated from the central nervous system using iridectomy scissors under a dissecting microscope.

Cell dissociation techniques

Cell dissociation methods which used in previous experiments were modified to get Purkinje cell with dendrites (Hockberger & Nam, 1994). First, lower concentration of enzyme without DNase was used to minimize any possible influence of enzymes. Secondly, cerebellar slices were used for better accessing to low concentration of enzyme instead of minced cerebellar pieces. The cerebellar vermis was sliced at 400 to 500 μm in thickness with tissue chopper. The slices were added to 10ml of antibiotic-isotonic solution containing papain (0.1 mg/ml, Worthington Biochemical Co., Freehold, NJ, USA) and incubated in a shaker waterbath with bubbling of 100% O_2 for 30 to 50 minutes at 22°C. After enzyme treatment, the slices were washed twice and transferred into a centrifuge tube (Falcon, USA) filled with a minimal essential medium (Gibco BRL Life Technologies, Inc., Gaithersburg, MD, USA). Afterward, the slice fragments were mechanically triturated with two or three fire-polished Pasteur pipettes, having a tip diameter 100~300 μm under the hood. Dissociated cells were plated on the poly-d-lysine coated coverslips (no. 2, Corning Inc. Corning, NY, USA) in a multiwell tissue culture plate for 30 min. Solution was changed to minimal essential medium containing 20% horse serum (Gibco) or bovine serum albumin (Irvine Scientific, USA). The plate was kept in incubator (NAPCO, model 6100, Tualatin, OR, USA) with 10% CO_2 at 37°C. Dissociated Purkinje neurons were easily recognized by their characteristic dendrites.

Electrophysiological procedures

Intracellular recordings were performed using whole-cell patch recording techniques as described by Hamill et al (1981). Coverslips containing isolated cells were mounted on the stage of a Nikon inverted microscope (Diaphot; Frank Fryer Co., Carpentersville, IL, USA) resting on a vibration-free, isolation table (Technical Manufacturing Corp., Peabody, MA, USA). Recording and puffing electrodes were made from glass microcapillary tubes (Dynalab, Rochester, NY, USA), pulled and polished using Narishige puller and microforge, and positioned using Narishige hydraulic micromanipulators (Medical Systems, Great Neck, NY, USA). Recording electrodes were not coated and had 2~3

MΩ of resistance in recording solutions. Solutions transferred to the electrodes using plastic cannula to avoid the effects of Cu²⁺ and Zn²⁺ released from metallic needles (Nam & Hockberger, 1992). After the gigaseal between the pipette and the membrane was established the membrane inside the pipette was broken by gentle negative pressure using suction. Spontaneous spikes were recorded from the cell with a good gigaseal without injection of any currents under current clamp. All recordings were performed at room temperature (18~22°C).

Spontaneous activity was stored on a videocassette recorder (model SLV-393, Sony, Japan) through a digital data interface (Instrutech Corp., model VR-10B, Great Neck, NY, USA). Currents were recorded using a List EPC-7 patch clamp amplifier (Medical Systems Corp., Greenvale, NY, USA), sampled at 3 kHz and digitized using a Labmaster 80 kHz A/D board interfaced with a Dell personal computer (System 210; Dell Computer Corp., Austin, TX, USA). The computer generated the voltage protocols and recorded the resulting currents using pClamp software (Axon Instruments, Burlingame, CA, USA) or Basic Fastlab software (Indec Corp., Sunnyvale, CA, USA). Data were displayed on a Tektronix storage oscilloscope (model 5113, Beaverton, OR, USA) during acquisition. Analysis of data were using Axotape (Axon Instruments), pClamp software (Axon Instruments) and Basic Fastlab software (Indec Co.). Data were plotted using a plotter (model 7470A, Hewlett Packard, USA) and a LaserWriter through AxoGraph software (Axon Instruments).

Composition of solutions

All solutions were prepared with water filtered through a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). A recording electrode was filled with internal saline without any blockers (normal internal saline) containing (mM) KAsp 130, Mg-ATP 2, CaCl₂ 1, EGTA 10, HEPES 10. The pH was adjusted to 7.3 with KOH. Cells on the coverslips were in the bath perfused with external saline. Spontaneous spikes were recorded in external saline contained (mM): NaCl 130, KCl 5.4, CaCl₂ 2, MgCl₂ 2, glucose 25, HEPES 10. The pH was adjusted to 7.3 with NaOH. Pharmacological agents were applied either in the bath or delivered locally via a nearby glass micropipet (2 μm opening). All chemicals was purchased

from Sigma chemical Co. (St. Louis, MO, USA). AP5 (Sigma) was solved in distilled water and diluted with external saline. (+) Bicuculline (Sigma) and DNQX (Tocris Cookson, St. Louis, MO, USA) were solved in chloroform and DMSO, respectively and diluted with external saline. Experimental solutions were prepared using at least 1000-fold dilutions of stock solutions.

Immunohistochemistry

Although isolated Purkinje cells with dendrites were easily identified with morphological characteristics, we need to confirm those cells without dendrites resulting from either cutting dendrites during dissociation or resorbing dendrites after dissociation. We identified the Purkinje cells using immunocytochemical staining techniques. Peroxidase-antiperoxidase (PAP) staining techniques was used using antisera and PAP complex supplied commercially (DAKO, Corp. and Vector Laboratories, Inc.). After recording, isolated cells on coverslips were washed in PBS for 5 min and fixed with paraformaldehyde for 30 min. Coverslips were washed three times with sterile PBS and then stored in aseptic tissue culture plates at 4°C until ready for staining. The staining procedure for polyclonal antibodies was performed at room temperature and was as follows.

- (1) 40 min incubation in blocking (non-immune) swine serum (DAKO) diluted 1:10 with PBS containing 0.5% Triton and 0.3% hydrogen peroxide;
- (2) three hour incubation in PBS-Triton solution containing rabbit polyclonal antibody in dark place; control tissues received PBS-Triton solution only;
- (3) ten min wash in PBS solution three times;
- (4) one hour incubation in swine anti-rabbit secondary antibody (DAKO) diluted 1:100 in PBS-Triton solution;
- (5) ten min wash in PBS solution three times;
- (6) one hour incubation in rabbit PAP antibody (DAKO) diluted 1:200 in PBS-Triton solution;
- (7) ten min wash in PBS solution three times;
- (8) stained for 5 to 30 min in 3-amino-9-ethylcarbazole (AEC) substrate solution (DAKO) in 0.1M acetate buffer containing 0.03% hydrogen peroxide;
- (9) ten min wash in PBS solution twice;
- (10) ten min wash in phosphate buffer solution twice;
- (11) mounted in a glycerol solution (DAKO) and covered with a glass coverslip.

Antibody against calbindin was used because it had been shown to stain Purkinje neurons selectively in

tissue sections from adult rat cerebellum (Enderlin et al, 1987). The diluted calbindin antibody (1:100,000) was used as the primary antibody. Positive stainings with anti-calbindin provided that the cell was a Purkinje neuron.

Digital photography and image processing

Photographs of immunostained tissue and isolated cells were taken with a video CCD camera interfaced

with a Perceptics image processing system. All photographs were frame-averaged and stored as digital images. Images were contrast-enhanced and sharpened using image processing software (BDS, Biological Detection Systems, Inc., Pittsburgh, PA, USA). Collages were created using BDS, Canvas 3 (Deneba Software, Miami, FL, USA) and Photoshop 2.5 (Adobe Systems Inc., Mountain View, CA, USA) software programs.

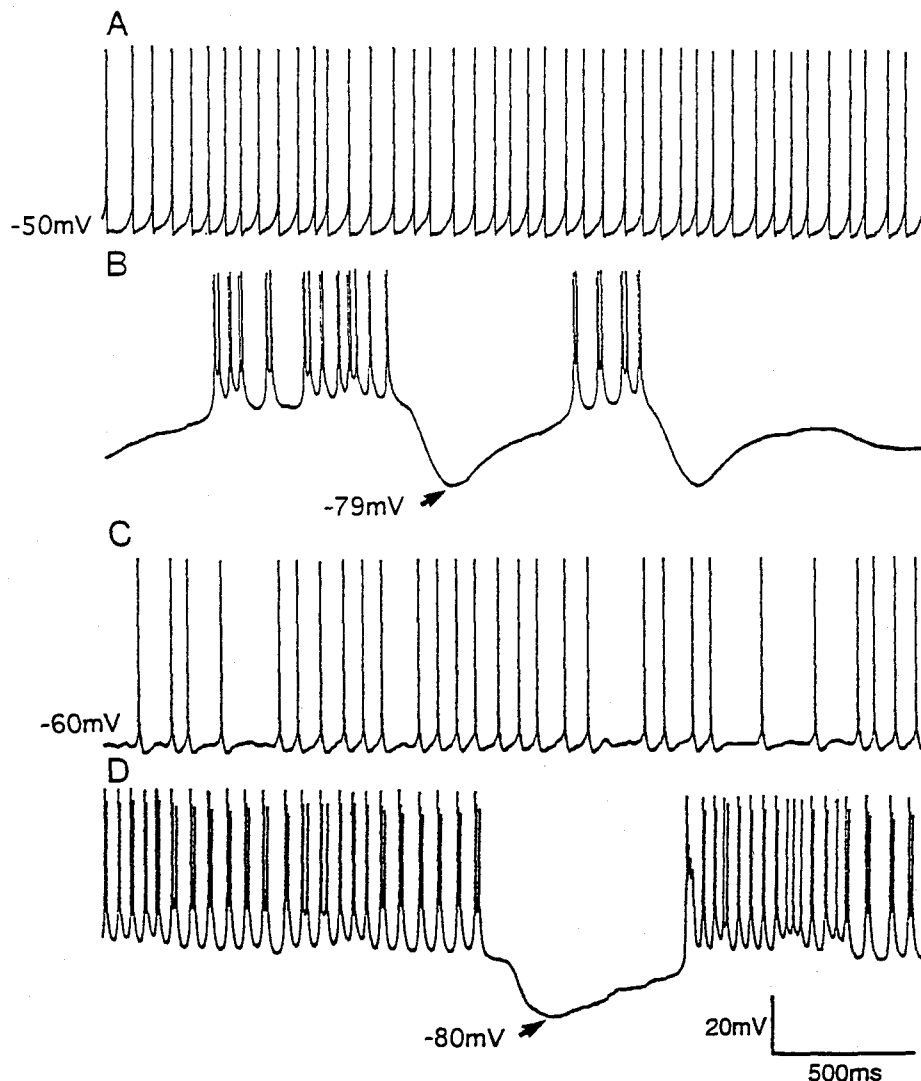


Fig. 1. Two types of spontaneous activity in acutely isolated cerebellar Purkinje cells. Two Purkinje cells (A: P14, B: P9) displayed two types of spontaneous activity, repetitive (A) and burst firing (B). Some Purkinje cells with burst firing at hyperpolarized potentials (D) displayed repetitive firing at depolarized membrane potential (C).

RESULTS

A tissue dissociation procedure used by Hockberger and his colleagues was modified because they did not record spontaneous activity from isolated Purkinje cells (Hockberger et al, 1989). The modified procedure included changes of enzyme treatment and mechanical trituration. 107 cells out of 165 Purkinje cells (65%) isolated with the modified procedure displayed spontaneous electrical activity in normal saline under current clamp. The percentage of sponta-

neously firing cells was dependent upon the isolation procedure. If cells were gently triturated, resulting in a high percentage of cells with dendrites and axons, more cells displayed spontaneous activity. However, if there was a small number of cells with dendrites and axons, the percentage of spontaneous activity decreased.

Two types of spontaneous activity, repetitive (70%) and burst (30%) firing, were observed in spontaneously firing Purkinje cells isolated from postnatal rats. Figure 1A and 1B show examples of each re-

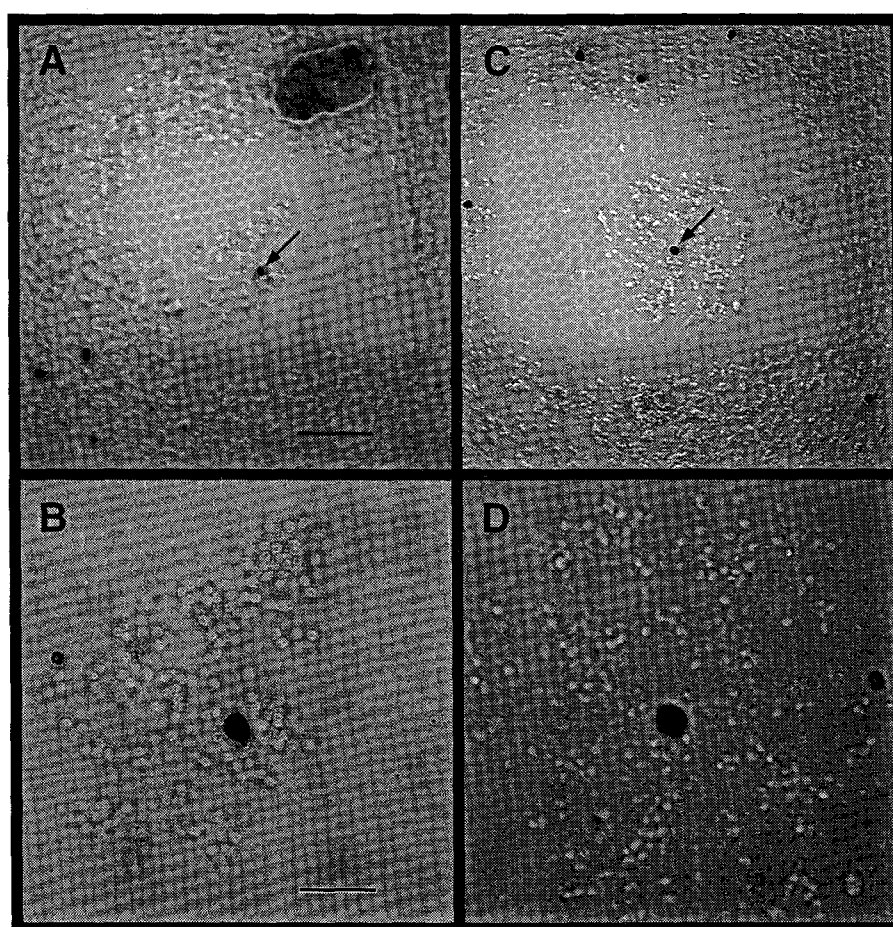


Fig. 2. Immunocytochemical identification of isolated cerebellar Purkinje cells with anti-calbindin after recording. A. Low power photomicrograph shows immunoreactivity of acutely isolated cells (P11) including the Purkinje cell from which spontaneous activity was recorded (arrow). B. Spontaneously firing cell stained with anti-calbindin was clearly shown on the center under high magnification. C. Low power photomicrograph shows immunoreactivity of cells (P11) including quiescent Purkinje cell (arrow) on another coverslip. D. Quiescent cell located on the center was also stained with anti-calbindin. B and D are higher magnification of A and C, respectively. Calibration bars are 250 μm (A,C) and 50 μm (B, D).

corded from different cells. Spontaneous repetitive firing activity was recorded from a cell at -50 mV of membrane potential (Fig. 1A). Spontaneous bursting activity was recorded from another cell at hyperpolarized membrane potential, -70 mV (Fig. 1B). When the cell with burst firing was depolarized, firing pattern was converted into repetitive firing. The cell with hyperpolarized resting membrane potential generated spontaneous burst firing (Fig. 1D). When the cell was held at depolarized level, -60 mV, it displayed repetitive firing (Fig. 1C).

Fig. 2 shows immunocytochemical staining of isolated cerebellar Purkinje cells (P11) using anti-calbindin after recording. A spontaneously firing cell was immunocytochemically stained with anti-calbindin after recording spontaneous activity in normal saline (Fig. 2A). High power photomicrograph shows clearer immunoreactivity of the marked spontaneously firing cell (Fig. 2B). Low power photomicrograph shows immunoreactivity of acutely isolated cerebellar cells in another coverslip containing a

marked quiescent cell (Fig. 2C). The quiescent cell was also immunocytochemically stained with anti-calbindin (Fig. 2D). These results demonstrated that both spontaneously and quiescent cells were cerebellar Purkinje cells.

Further studies were performed to investigate whether acutely isolated Purkinje cell can generate spontaneous activity without any synaptic inputs. Although acutely isolated Purkinje cells generated spontaneous activity in previous studies, possible synaptic inputs could not be excluded because of the limited resolution of a light microscope. Scanning electron microscopy of Purkinje cells isolated during the second week of birth demonstrated Purkinje cells with many fine processes touching granular cells, excitatory neurons (Hockberger et al, 1994). Two different methods, physical and pharmacological, were used to remove any possible synaptic inputs in the present study. For these experiments, a small number of cells were plated on coverslips to reduce the possibility of connections between cells.

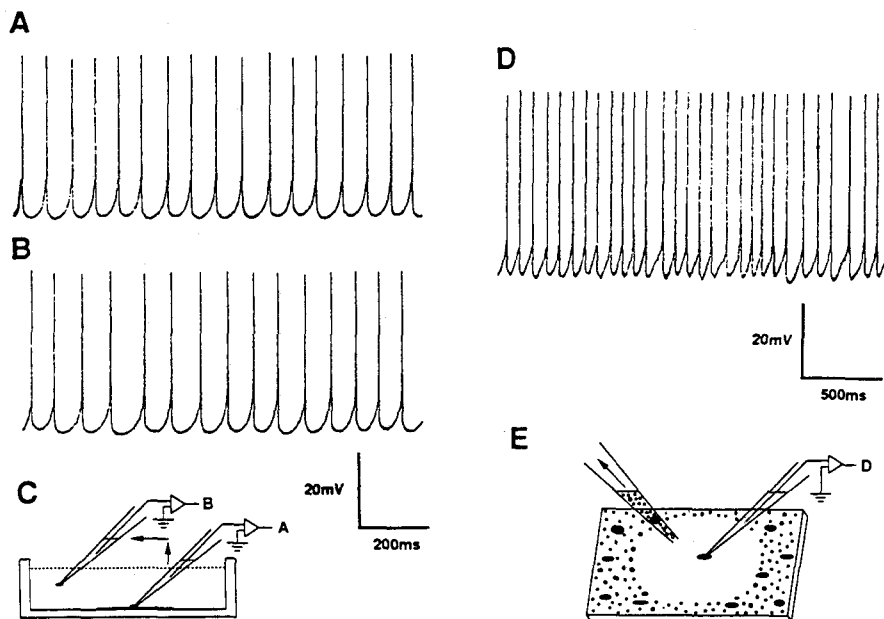


Fig. 3. Spontaneous activity recorded from physically isolated Purkinje cells. A. An acutely isolated Purkinje cell with dendrites (P14) displayed spontaneous activity. To eliminate any possible synaptic connections the cell was lifted off the coverslip and moved away as diagrammed in C. This cell still displayed spontaneous activity after lifting off (B). In another method of physical isolation (E) the region around a cell was scraped to destroy any possible connections before electrical activity was recorded. A Purkinje cell (P10) isolated in this way displayed spontaneous activity at resting membrane potential, -52 mV under current clamp (D).

First, physical isolation was performed in two ways: the cell was lifted off the coverslip and moved away from the site during recording (Fig. 3C) or the area around cells was scraped and cleaned (Fig. 3E). Since most of cells were not detached from the coverslip in the first approach, the period of time was reduced from 30 min to 10 min after plating cells on coverslips, resulting in a weaker cell-coverslip adhesion. Fig. 3 shows spontaneous activity recorded from isolated Purkinje cell (P14) before (A) and after (B) lifting. There was slight decrease in spike frequency

from 15.6 Hz to 14.4 Hz and no change in amplitude, 70 mV. This cell maintained firing for longer than 30 min after lifting. Although lifting cells off coverslips without losing the seal was not easy, the lifted cells kept firing until the cell was leaky or dead (N=3). In the second approach the region around cell was scraped to sever any possible connections (Fig. 3E). The region around cell was scraped before instead of during recording so I could scrape all around the cell (360°). Thirteen out of fourteen cells isolated with this procedure displayed spontaneous activity.

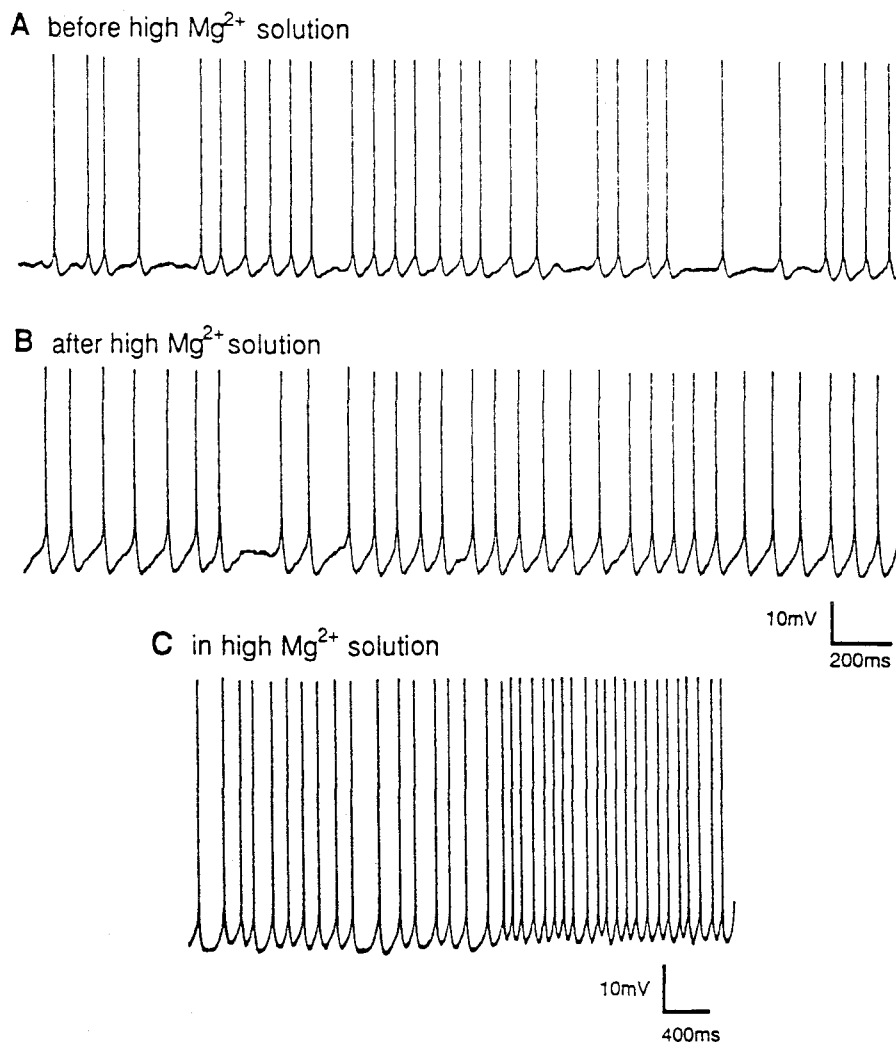


Fig. 4. Spontaneous activity recorded from acutely isolated Purkinje cells in high Mg^{2+} external saline. A. A Purkinje cell (P14) displayed spontaneous activity in normal saline. B. When the bath was changed to high Mg^{2+} external saline (10 mM) spontaneous activity was also recorded from the same cell. Another Purkinje cell (P11) displayed spontaneous activity at resting membrane potential, -56 mV in high Mg^{2+} external saline (C).

One example of spontaneous activity recorded from isolated Purkinje cell (P10) is shown in Fig. 3D.

Second, general synaptic blockers or antagonists of the neurotransmitter were used in pharmacological isolation method. High Mg^{2+} saline (10 mM) was used for general synaptic blocker. Fig. 4 shows an effect of high Mg^{2+} saline on spontaneous activity generation. Spontaneous spikes were recorded from acutely isolated Purkinje cell (P14) before (Fig. 4A) and after (Fig. 4B) changing the bath to high Mg^{2+} saline (10 mM) or from cells bathed in high Mg^{2+} saline from the start (Fig. 4C). The high Mg^{2+} saline did not block spike generation even though spike frequency and amplitude were slightly changed. This subtle change of spike frequency occurred as membrane potential changed because of voltage dependence of spike frequency.

In addition to general synaptic blocker, the effects of antagonists of excitatory and inhibitory neurotransmitters such as DNQX, AP-5, and bicuculline on

the spike generation were also examined. Fig. 5 shows spontaneous activity before (A) and after (B) applying DNQX which is known as antagonist of non-NMDA receptor. Acutely isolated Purkinje cell (P14) displayed spontaneous activity with 27 Hz of frequency, 85 mV of amplitude at resting membrane potential, -54 mV (Fig. 5A). Amplitude and frequency of spikes were not changed after applying DNQX ($50 \mu M$) to the bath (Fig. 5B). Although there is a controversy about an existence of NMDA receptor in rat cerebellar Purkinje cell, an effect of antagonist of NMDA receptor, AP-5 was tested (C: before, D: after applying). Spontaneous spikes were recorded from different cell (P13). Frequency and amplitude of spikes were 17 Hz, 80 mV, respectively (Fig. 5C). Spike frequency was not changed after applying AP-5 ($50 \mu M$) even though amplitude of spikes decreased slightly from -80 to -75 mV (Fig. 5D). GABA antagonist was applied to eliminate any influence of in-

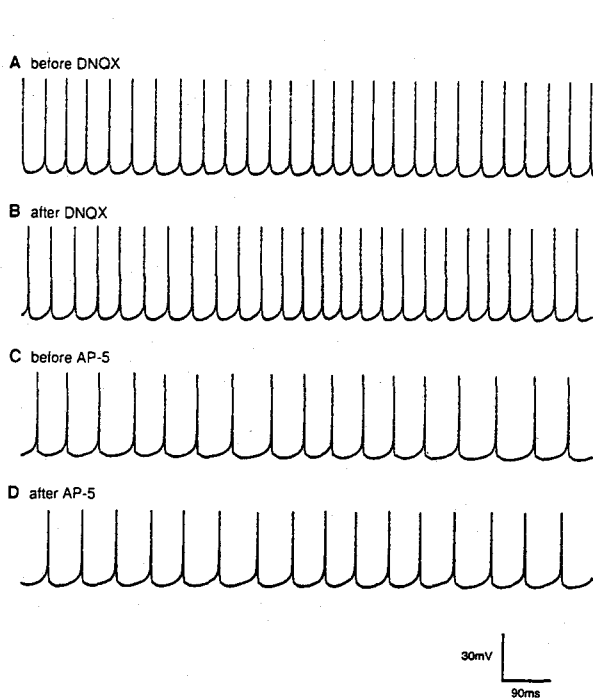


Fig. 5. The effects of excitatory neurotransmitter antagonists on the generation of spontaneous activity. A. Spontaneous activity was recorded from an acutely isolated Purkinje cell (P14) under current clamp. B. DNQX ($50 \mu M$) was applied to the bath during recording of spontaneous activity. Another isolated Purkinje cell (P13) displayed spontaneous activity before (C) and after (D) applying AP-5 ($50 \mu M$) to the bath.

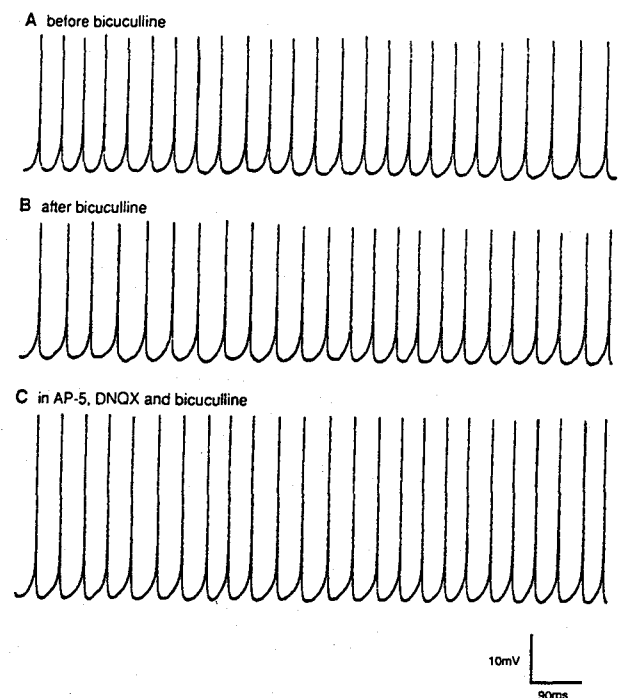


Fig. 6. The effects of either inhibitory or both excitatory and inhibitory neurotransmitter antagonists on the generation of spontaneous activity. Spontaneous activity was recorded from an isolated Purkinje cell (P14) before (A) and after (B) applying bicuculline ($10 \mu M$) to the bath. C. Another isolated Purkinje cell (P14) displayed spontaneous activity in the presence of $50 \mu M$ AP-5, $50 \mu M$ DNQX, and $10 \mu M$ bicuculline.

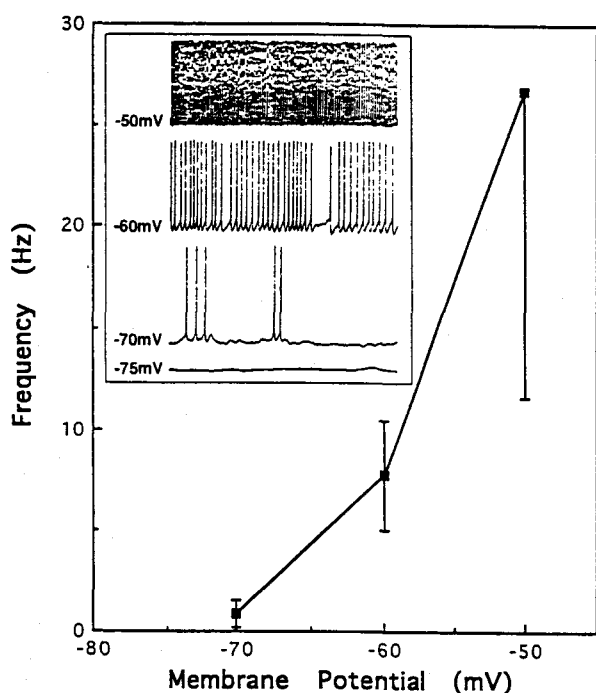


Fig. 7. Voltage dependency of spike frequency. Spike frequency of acutely isolated Purkinje cells was changed as membrane potential changed under current clamp. Each data point is the average frequency of spikes recorded from five cells at different membrane potentials. Error bars indicate standard deviations. The inset shows an example of voltage dependency of spike frequency.

hibitory synapse during recording spontaneous activity. Acutely isolated Purkinje cell (P14) displayed spontaneous activity with 19 Hz of frequency and 65 mV of amplitude at resting membrane potential -48 mV (Fig. 6A). Spike frequency was not changed after applying bicuculline ($10 \mu\text{M}$) to the bath (Fig. 6B). Furthermore, isolated Purkinje cell (P14) can still generate spontaneous activity in the presence of all three antagonists of excitatory and inhibitory neurotransmitters, DNQX, AP-5 and bicuculline (Fig. 6C).

Traditional methods using electrophysiological recording technique have been used to verify that the source of spontaneous activity is endogenous. One of the methods, a voltage dependency of spike frequency, was tested in this experiments. Membrane potential was changed during the recording of spontaneous activity. Average frequency of spike in five cells was 7.7 Hz at -60 mV of membrane potential (Fig. 7). When cells were held at a more depolarized level, -50

mV, the spike frequency increased from 7.7 Hz to 26.7 Hz. The spike frequency was decreased from 7.7 Hz to 0.9 Hz at hyperpolarized membrane potential, -70 mV. Sufficient hyperpolarizing membrane potential terminated spontaneous activities completely and there was no evidence of synaptic activity (Fig. 7, inset). Another electrophysiological test for spontaneous activity, a rhythm reset test, is useful in burst firing cells. However, the test could not be examined because burst firing in isolated Purkinje cells was irregular.

DISCUSSION

In this report it was determined that cerebellar Purkinje cells dissociated from postnatal rats display spontaneous electrical activity in the absence of synaptic inputs. The cell isolation procedure was very important to detect this activity. When the majority of Purkinje cells were isolated without dendrites, they did not display spontaneous activity. This might explain why Hockberger et al (1989) did not observe spontaneous activity in Purkinje cells isolated from animals of the same age. By modifying their dissociation procedures to obtain more Purkinje cells with dendrites, spontaneous activity was recorded from isolated Purkinje cells. However, not all Purkinje cells examined in this study displayed spontaneous electrical activity. About one third of Purkinje cells isolated using the modified procedure did not display spontaneous electrical activity in normal saline under current clamp but two thirds did. Although there was no morphological difference between quiescent and spontaneously firing cells, both types of cells displayed positive immunoreactivity of antibody against calbindin identifying as Purkinje cells. Previous experiment already determined that positively immunostained cerebellar cells with anti-calbindin were identified as Purkinje cells (Hockberger et al, 1994).

The discrepancy of the spike generation between two types of Purkinje cells was probably due to two possibilities. One is that Purkinje cell dendrites might play an important role in generating spontaneous activity. If they do, intrinsic properties of their membrane could differ from those of membrane on Purkinje cell body. This possibility is supported by several results from molecular biological studies. For example, type I alpha subunit of sodium channels was segregated in

rat Purkinje cell bodies whereas type II alpha subunit was located in molecular layer containing dendrites of Purkinje cells (Westenbroek et al, 1989; Mandel, 1992). The other possibility is that Purkinje cells without dendrites were too weak to generate spontaneous activity due to enzymatical and mechanical damage. This possibility is unlikely to explain the discrepancy in the spike generation since Purkinje cells with or without dendrites displayed sodium or calcium currents in previous experiments (Nam & Hockberger, 1992; Hockberger & Nam, 1994). Purkinje cells with or without spontaneous activity were observed in other types of experiments *in vitro*. For example, half of cultured Purkinje cells examined in the presence of high Mg^{2+} displayed spontaneous activity but half did not (Gahwiler et al, 1973). Quiescent and several types of firing patterns of Purkinje cells were also observed in slice experiments (Chang et al, 1993).

The mechanisms for the generation of these spontaneous activities can be divided into two broad categories, endogenous and exogenous (Moffett, 1977; Friesen & Stent, 1978). Some neurons can generate spontaneous electrical activity through electrical or chemical synaptic inputs from an endogenously active neuron or network of neurons. Spontaneous bursting activities generated by synaptic interactions among a group of neurons have been found in a number of systems including swimming system in *Tritonia* (Getting et al, 1980), locomotion and mastication in vertebrate animals (Grillner, 1975; Lund & Enomoto, 1988). In contrast endogenously firing cells display spontaneous activity in the absence of electrical or chemical synaptic inputs. The spontaneous activity of endogenously firing cells results from their intrinsic membrane properties. Endogenous electrical activity has been observed in both invertebrate and vertebrate neurons. For example, surgically or enzymatically isolated *Aplysia* neurons (R15) displayed spontaneous electrical activity (Alving, 1968; Chen et al, 1971). Hippocampal pyramidal neurons also displayed spontaneous activity in the presence of synaptic blocker (Hablitz & Johnston, 1981). Furthermore this spontaneous activity was recorded from isolated hippocampal pyramidal neurons (Wong et al, 1986).

In the case of repetitive firing of cerebellar Purkinje cells, I determined that the spontaneous firing was due to endogenous membrane properties since it persisted under conditions that eliminated synaptic

inputs. Data in this study demonstrated that dissociated Purkinje cells with dendrites displayed spontaneous activity like other endogenously firing cells. Spontaneous activity of dissociated Purkinje cells was not eliminated by physical further isolations such as detaching cell from coverslip or scraping around cell. Furthermore dissociated Purkinje cells displayed spontaneous activity in the presence of either general synaptic blocker like high Mg^{2+} or specific synaptic blockers, antagonists of excitatory and inhibitory neurotransmitters. DNQX, an antagonist of non-NMDA receptor, known to exist in cerebellar Purkinje cells did not block spontaneous activity of the cells. Another antagonist of excitatory neurotransmitter NMDA, AP-5 did not eliminate the spontaneous electrical activity of dissociated Purkinje cells. Bicuculline, an antagonist of an inhibitory neurotransmitter GABA did not affect the generation of spontaneous activity in dissociated Purkinje cells, suggesting that inhibitory synaptic transmission did not involve in spike generation. These results are consistent with those in rat Purkinje cell *in vivo* and *in vitro* experiments. Purkinje cells displayed spontaneous activity either before synapses formed (P1) or after elimination of the excitatory pathway *in vivo* (Woodward et al, 1974; Crepel et al, 1981). High Mg^{2+} saline did not eliminate spontaneous activity of cultured Purkinje cells (Gahwiler et al, 1973). Spontaneous electrical activity recorded from adult Purkinje cells in cerebellar slices persisted in the presence of TTX (Llinas & Sugimori, 1980). These results indicate that cerebellar Purkinje cells generate spontaneous activity by intrinsic membrane properties.

In addition to several lines of evidence indicating that spontaneous activity of Purkinje cells was generated by endogenous membrane properties using physical or pharmacological methods, the cells showed the voltage dependency of spike frequency. Spike frequency of spontaneous activity recorded from dissociated Purkinje cells was changed depending upon membrane potentials. That is, the frequency of firing is increased by depolarizing current injection, while it is decreased by hyperpolarizing current. This pattern was also observed in other endogenously firing cell. For example, the spike frequency of endogenously firing neuron (R15) of *Aplysia* was decreased when hyperpolarizing current was injected. With even greater hyperpolarizing current, firing spike could be terminated (Arvanitaki & Chalazonitis, 1968).

Two types of spontaneously firing patterns, repetitive and burst firing were observed in Purkinje cells in the same condition. Morphological characteristics such as size and shape of repetitive firing cells did not differ from those in bursting firing cells. Two patterns of spontaneous activity in cerebellar Purkinje cells were also observed in other type of experiment. Repetitive and burst firing activities were recorded from Purkinje cells of guinea pig in slice experiment (Llinas & Sugimori, 1980). However, burst firing with long plateau potentials and large afterhyperpolarization was not observed in cultured Purkinje cells (Gruol & Franklin, 1987). The discrepancy was probably due to change of membrane properties in cultured cells. Channel expression of the cell membrane was affected by culture media. For example transient potassium current (I_A) was recorded from cerebellar Purkinje cells cultured in only L15 (Leibovitz) medium (Bossu et al, 1988). Two types of firing patterns were also observed in other vertebrate neurons including thalamic neurons (Jahnsen & Llinas, 1984). In repetitive and burst firing activity, Purkinje cells displayed spontaneous firing of doublet and complex spikes in the middle of transition from bursting to repetitive firing. These doublet and complex spikes in dissociated Purkinje cells are very similar to those in cultured rat Purkinje cells (Gruol & Franklin, 1987). Cultured Purkinje cells displayed repetitive firing of simple or doublet or complex spikes, and burst firing of three to four spikes even though they did not display burst firing with long plateau potentials and afterhyperpolarization. Simple and complex spikes were used for identifying that a recording electrode was located at Purkinje cell or Purkinje cell layer in vivo experiments (Thach, 1967). In slice experiments, simple spikes and rhythmic bursting activity were recorded from Purkinje neurons of guinea pig (Hounsgaard, 1979; Llinas & Sugimori, 1980).

Although the functional role of the spontaneous electrical activity of Purkinje neurons has not been clearly understood, the activity of the sole output from the cerebellar cortex may play an important role in the timing properties of motor execution, and in nonmotor functions as well. It has been recently known that the cerebellum not only controls motor function but also involves motor learning and memory and nonmotor functions. Recent studies using positron emission tomography or magnetic resonance imaging techniques demonstrated that the cerebellum is

involved in nonmotor functions such as cognitive processing (Kim et al, 1994), sensory discrimination (Gao et al, 1996), and verbal memory (Andreasen et al, 1996). Further attention and motor performance independently activate distinct cerebellar regions (Allen et al, 1997). The left quadrangular lobule and the left superior semilunar lobule are involved in attention, whereas the right anterior vermis, the right central lobule and the right anterior quadrangular lobule are involved in motor task. These results suggest that the spontaneous electrical activity of the cerebellar neurons, especially Purkinje cells may play an important role in both motor function and a variety of nonmotor functions.

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