Comparative Effects of Methylmercury on Parallel-Fiber and Climbing-Fiber Responses of Rat Cerebellar Slices

YUKUN YUAN and WILLIAM D. ATCHISON

Department of Pharmacology and Toxicology, Neuroscience Program and Institute for Environmental Toxicology, Michigan State University, East Lansing, Michigan

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ABSTRACT

The environmental neurotoxicant methylmercury (MeHg) causes profound disruption of cerebellar function. Previous studies have shown that acute exposure to MeHg impairs synaptic transmission in both the peripheral and central nervous systems. However, the effects of MeHg on cerebellar synaptic function have never been examined. In the present study, effects of acute exposure to MeHg on synaptic transmission between parallel fibers or climbing fibers and Purkinje cells were compared in 300- to 350-μm cerebellar slices using extracellular and intracellular microelectrode-recording techniques. Field potentials of parallel-fiber volleys (PFVs) and the associated postsynaptic responses (PSRs) were recorded in the molecular layer by stimulating the parallel fibers in transverse cerebellar slices. The climbing-fiber responses were also recorded in the molecular layer by stimulating white matter in sagittal cerebellar slices. At 20, 100, and 500 μM, MeHg reduced the amplitude of both PFVs and the associated PSRs to complete block, however, it blocked PSRs more rapidly than PFVs. MeHg also decreased the amplitudes of climbing-fiber responses to complete block. For all responses, an initial increase in amplitude preceded MeHg-induced suppression. Intracellular recordings of excitatory postsynaptic potentials of Purkinje cells were compared before and after MeHg. At 100 μM and 20 μM, MeHg blocked the Na⁺-dependent, fast somatic spikes and Ca²⁺-dependent, slow dendritic spike bursts. MeHg also hyperpolarized and then depolarized Purkinje cell membranes, suppressed current conduction from parallel fibers or climbing fibers to dendrites of Purkinje cells, and blocked synaptically activated local responses. MeHg switched the pattern of repetitive firing of Purkinje cells generated spontaneously or by depolarizing current injection at Purkinje cell soma from predominantly Na⁺-dependent, fast somatic spikes to predominantly Ca²⁺-dependent, low amplitude, slow dendritic spike bursts. Thus, acute exposure to MeHg causes a complex pattern of effects on cerebellar synaptic transmission, with apparent actions on both neuronal excitability and chemical synaptic transmission.

The environmental neurotoxicant methylmercury (MeHg) causes disruptions of both sensory and motor function after acute or chronic exposure (Takeuchi et al., 1962; Bakir et al., 1973). Among the brain regions most prominently affected by MeHg is the cerebellum. During chronic exposure, MeHg accumulates most in the cerebellum, particularly in the Purkinje and Golgi cells of the granular layer, and to a lesser extent in the granule cells, stellate cells, and basket cells (Glomski and Brody, 1971; Chang, 1977; Møller-Madsen, 1990, 1991; Leyshon-Sørland et al., 1994). Pathological examination of patients and experimental animals with acute and chronic MeHg poisoning indicates that the granule cells are particularly sensitive to MeHg (Hunter and Russell, 1954; Takeuchi et al., 1962; Chang, 1977; Syversen, 1981). In MeHg poisoning, especially in chronic cases, there is a characteristic atrophy of the cerebellar cortex, particularly the granular layer in the lateral lobes and in the vermis, due to extensive loss of granule cells (Hunter and Russell, 1954; Takeuchi et al., 1962; Chang, 1977). The basket cells, and climbing and parallel fibers are also severely involved. Purkinje cells are more resistant, but are also typically affected in chronic cases (Hunter and Russell, 1954; Takeuchi et al., 1962; Chang, 1977). The interactions of MeHg with cerebellar neurons are likely to be responsible for the motor deficits caused by acute and chronic exposure to MeHg; however, the
mechanism by which MeHg disrupts cerebellar function is not yet known.

Like other toxic heavy metals, MeHg has pronounced effects on synaptic function. Acute bath application of MeHg disrupts neuronal membrane excitability and synaptic transmission in the CA1 region of hippocampal slices in a concentration- and time-dependent manner (Yuan and Atchison, 1993, 1995, 1997). MeHg appears to act at multiple sites and by multiple mechanisms to cause these effects. It blocks excitatory and inhibitory synaptic transmission as well as antidromically-activated responses. It hyperpolarizes and then depolarizes CA1 pyramidal cell membranes and it may also affect presynaptic release of neurotransmitters.

The effects of MeHg on cerebellar synaptic transmission have not yet been characterized, although several studies have characterized cellular effects of MeHg on cerebellar granule neurons in isolation. In vitro exposure to MeHg blocks depolarization-dependent influx of $^{45}\text{Ca}^{2+}$ into rat cerebellar synaptosomes (Yan and Atchison, 1996), reduces the amplitude of granule cell $\text{K}^{+}$ and $\text{Ca}^{2+}$ currents (Sirois et al., 1995; Sirois and Atchison, 1996), increases granule cell $[\text{Ca}^{2+}]$, (Marty and Atchison, 1997), and induces cell death (Marty and Atchison, 1998). All of these actions could influence synaptic transmission in the cerebellum. The unique architecture of the cerebellar cortex suggests that Purkinje cells are a key element in the cerebellar synaptic circuits because they receive and integrate synaptic inputs from both parallel and climbing fibers, and their axons are the only output leaving the cerebellar cortex for the deep cerebellar nuclei. Thus, we sought to determine whether MeHg affects synaptic transmission between the parallel or climbing fibers and Purkinje cells, and in particular, whether transmission through synapses originating in granule cells (parallel fibers) might be affected preferentially by MeHg given its apparent predilection for inducing granule cell toxicity.

Materials and Methods

Materials. Methylmercuric chloride (ICN Biomedicals, Inc., Costa Mesa, CA) was dissolved in deionized water to a concentration of 5 mM to serve as stock solution. The applied solutions (20–100 $\mu$M) were diluted with modified artificial cerebrospinal fluid (ACSF) consisting of 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 1.25 mM KH$_2$PO$_4$, 26 mM NaHCO$_3$, and 20 mM D-glucose (pH to 7.4 at room temperature just before superfusion). MeHg and other chemicals were applied continuously to slices by bath application at a rate of 1.2 to 1.5 ml/min using a Gilson infusion pump (Gilson Medical Electronics, Inc., Middleton, WI). 6,7-Dinitroquinoxaline-2,3-dione (DNQX) and amino-5-phosphonopentanoic acid were purchased from Sigma Chemical Co. (St. Louis, MO). DNQX was dissolved first in dimethyl sulfoxide and then diluted further with modified ACSF. The final concentration of dimethyl sulfoxide in the applied solution was less than 0.02% (v/v), which has no significant effects on synaptic transmission (results not shown).

Preparation of Cerebellar Slices. Cerebellar slices were prepared using methods modified slightly from those reported previously (Llinás and Sugimori, 1980a,b; Konnerth et al., 1990; Ilano et al., 1991). In brief, the cerebellum was removed quickly from the brain of a Sprague-Dawley rat (either gender, 15–20 days postnatal) and immersed immediately in cold, oxygenated modified ACSF for 1 to 3 min. A portion of vermis (for sagittal slices) isolated by two sagittal cuts, or the whole cerebellum (for transverse slices) was transferred to the recording chamber and the remaining slices were incubated in a holding chamber for later use if needed. The entire process, from decapitating the rat to transferring the slices to the recording or holding chamber, was finished in less than 10 min at 4°C. The slice in the recording chamber was incubated continuously with freshly oxygenated (saturated with 95% O$_2$/5% CO$_2$) ACSF for at least 60 min before the electrophysiological recordings began. All experiments were conducted at room temperature of 22–25°C. Only one slice per rat was used for any given experiment.

Electrophysiological Procedures. The arrangement of stimulating and recording electrodes for transverse and sagittal slices is shown schematically in Fig. 1. A concentric bipolar metal electrode or monopolar tungsten electrode (3 MΩ; FHC, Inc.) was used as the stimulation electrode. Borosilicated glass microelectrodes (i.d. 1.0 mm, i.d. 0.5 mm; WPI, Inc., Sarasota, FL) filled with ACSF (5–15 MΩ impedance) or 3 M potassium acetate (60–80 MΩ impedance) were used as extracellular or intracellular recording electrodes, respectively. Conventional extracellular recordings were made in the molecular layer of the cerebellar cortex. To record extracellular responses of Purkinje cells to local extracellular activation of the parallel fibers, the stimulating electrode was positioned on the surface of the molecular layer of a transverse slice (Fig. 1, S1), just below the pia, and a recording electrode (Fig. 1, R1) was positioned on the same track along which parallel fibers travel, so-called “on beam”, in the molecular layer. As depicted, the typical extracellular response evoked by activation of parallel fibers consists of an initial triphasic potential with positive-negative-positive components followed by another prolonged negative potential. The initial triphasic component corresponds to the current generated by action potentials propagating along parallel fibers, defined as the parallel fiber volley (PFV; Fig. 1). The prolonged negative potential corresponds primarily to the postsynaptic excitatory potentials evoked by glutamate released from parallel fibers onto the molecular layer dendrites of Purkinje cells. It is defined as the postsynaptic response (PSR; Fig. 1), because it can be blocked reversibly with the glutamate N-methyl-D-aspartate-type receptor antagonist DNQX (Salin et al., 1996). To record field responses of Purkinje cells to activation of climbing fibers, the stimulating electrode was positioned on the white matter immediately at the base of the folium in a parasagittal slice (Fig. 1, S2); a recording electrode was positioned in the molecular layer (Fig. 1, R2). The field potentials evoked by stimulation of climbing fibers are also glutamate-evoked responses because they were blocked by DNQX (results not shown). Normally, it required 20 to 30 min for the amplitudes of these field potentials to stabilize before starting experiments. In most recordings, amplitudes of the field responses increased within the first 30 min after establishing the recordings in the absence of any treatment.

For intracellular recordings of parallel- or climbing-fiber excitatory postsynaptic potentials (PF-EPSPs or CF-EPSPs), the positions of stimulating electrodes were similar to those for extracellular recordings; however, the recording electrodes were positioned in the soma of an identified Purkinje cell with an Olympus BHWI upright microscope (Olympus Optical Corp. Ltd., Tokyo, Japan) equipped with Naromarski optics and $\times$10 and $\times$40 water-immersion objectives. The stimulus pulses were generated using a Grass S88 stimulator and isolated using a Grass SIU5 (Grass, Inc., Quincy, MA) at 0.15 Hz and 0.1-ma duration and an initial intensity that produced approximately 50 to 60% of the maximum response for a given slice. Recorded signals were amplified (Axoc-lamp-2; Axon Instruments Inc., Foster City, CA), displayed on a
2090-3 digital oscilloscope (Nicolet Instrument Corp., Verona, WI), and recorded simultaneously for later analysis to a 5X86 personal computer at a digital sampling interval of 0.2 (for individual spikes) or 1.0 ms (for repetitive firing) using the SCAN synaptic current analysis program V3.0 developed by Dr. John Dempster (University of Strathclyde, Scotland). The responses of Purkinje cells to the activation of parallel fibers and climbing fibers were identified further based on their distinctive electrophysiological properties (Llinares and Sugimori, 1980a; Crepel et al., 1981; Stuart and Häusser, 1994). The responses, recorded extracellularly or intracellularly and generated by activation of parallel fibers, are graded events, whereas responses evoked by stimulating climbing fibers are so-called all or none complex spikes regardless of the stimulus intensity used. Direct depolarization of Purkinje cells by injection of 500- to 1000-ms positive current pulses at the threshold intensity using the recording electrode in the soma generates Purkinje cell-specific repetitive spikes.

Statistical Analysis. Data were collected continuously before and during application of MeHg and analyzed statistically using Student’s paired t test or one-way analysis of variance for time-dependent measures. Dunnett’s procedure was used for post hoc comparisons. Values were considered statistically significant at $P < .05$. Experiments were replicated a minimum of five times.

Results

Comparative Effects of MeHg on Field Potentials Evoked by Stimulating Parallel Fibers or Climbing Fibers. The general effects of MeHg on field potentials recorded from the molecular layer of transverse and sagittal cerebellar slices are shown as representative tracings in Fig. 2. Acute bath application of 20, 100, and 500 $\mu$M MeHg to transverse slices caused a concentration- and time-dependent biphasic effect on the PFV and associated PSRs generated by stimulation of the parallel fibers. Initially, the amplitudes of PFVs and PSRs were increased (Fig. 2; data for 20 and 500 $\mu$M are not shown to simplify the figure). This effect was followed by a gradual reduction in amplitude of the response. However, as shown in Fig. 2, MeHg did not affect all responses with a similar time course. At 30 min after exposure to 100 MeHg, the PSR components were blocked completely, whereas the PFV components remained essentially unchanged. As shown in Fig. 2, for sagittal slices, 100 $\mu$M MeHg caused qualitatively similar effects. Initially, MeHg stimulated the amplitude of field potentials evoked by stimulation of climbing fibers before blocking them.
The comparative time courses of effects of MeHg on PFVs, PSRs, and climbing-fiber responses (CFRs) are summarized in Fig. 3. The time courses for individual experiments are obscured by pooling the data. The percentages of peak increases in amplitudes of PFVs and PSRs, averaged from each individual experiment, were $157 \pm 17\%$ and $128 \pm 8\%$ of control for $100 \mu M$ MeHg and $211 \pm 25\%$ and $178 \pm 19\%$ of control for $20 \mu M$ MeHg, respectively. The mean times to peak stimulation of PFVs and PSRs averaged from each individual experiment were $24 \pm 4$ min and $9 \pm 2$ min for $100 \mu M$ MeHg and $91 \pm 22$ min and $49 \pm 12$ min for $20 \mu M$ MeHg, respectively. As time of exposure of slices to MeHg increased, both PFV and PSR amplitudes were reduced progressively until complete block occurred. Figure 3 substantiates the observation that MeHg blocked PSRs more rapidly than it did PFVs. The mean times to block PSRs by $500 \mu M$, $100 \mu M$, and $20 \mu M$ MeHg were $6 \pm 0.5$, $32 \pm 4$, and $101 \pm 24$ min, respectively; the mean times to block PFVs by $500 \mu M$, $100 \mu M$, and $20 \mu M$ MeHg were $10 \pm 0.5$, $51 \pm 5$, and $184 \pm 27$ min, respectively. Differences between times to MeHg-induced block of PSRs and PFVs were statistically significant ($p < .05$). Thus, the glutamate-mediated PSRs appear to be more sensitive to MeHg than were the presynaptic parallel-fiber responses.

The percentages of peak increases in CFR amplitudes stimulated by $100 \mu M$ and $20 \mu M$ MeHg were $128 \pm 6\%$ and $131 \pm 7\%$ of control ($p < .05$), respectively. Times to block of CFRs by $100 \mu M$ and $20 \mu M$ MeHg were $45 \pm 3$ and $115 \pm 18$ min, respectively. These values are similar to those for MeHg-induced block of the PSRs, and appear to be more rapid than those for MeHg-induced block of the PFVs, although the differences were not statistically significant due likely to the unpaired nature of the comparison. (The PSR and PFV were measured simultaneously, and thus are paired measurements.) Thus, MeHg blocked glutamate-mediated postsynaptic responses activated either by stimulating parallel or climbing fibers with similar time courses, suggesting that MeHg may affect either the postsynaptic glutamate receptors or transmitter release from parallel or climbing fiber terminals to block synaptic transmission by these two pathways.
Effects of MeHg on PF-EPSPs and CF-EPSPs. To explore the mechanisms underlying the effects of MeHg on field potentials recorded in the molecular layer by activation of parallel fibers or climbing fibers, intracellular recording techniques were applied to the Purkinje cells to examine effects of MeHg on PF-EPSPs, CF-EPSPs, and resting membrane potentials. PF-EPSPs were graded responses with a range of 1.5- to 2.3-ms latencies from the stimulus artifact to the onset of EPSPs, depending on the stimulus intensity and the distance between the stimulating and recording electrodes. After exposure to 100 μM and 20 μM MeHg, these latencies were prolonged, suggesting that impulse conduction from the parallel fibers to Purkinje cells was slowed. Unlike the biphasic effects of MeHg on field potentials, 100 μM and 20 μM MeHg typically blocked the PF-EPSPs without causing an early increase in EPSP amplitude, although a transient slight increase in amplitude of EPSPs occurred in some slices before suppression of the EPSPs (results not shown). Times to block of EPSPs by 100 μM and 20 μM MeHg were 33 ± 7 and 57 ± 6 min, respectively. However, as shown in Fig. 4 (top), the PFVs appeared less sensitive to MeHg than did PF-EPSPs because PFV amplitudes remained essentially unchanged when EPSPs were reduced significantly (10 min) or blocked completely by MeHg (100 μM). This was consistent with results obtained from extracellular recordings in that PFVs were less sensitive to MeHg than were the associated PSRs. The early block of EPSPs by MeHg could be restored in part by increasing stimulus intensity (Fig. 4, left). However, subsequently, EPSP amplitude was reduced to block.

Stimulation of climbing fibers in sagittal cerebellar slices in the absence of MeHg generated a full antidromically activated action potential followed by a typical all or none complex spike response or CF-EPSP (Fig. 4, right). The complex spikes consist of several small spikes superimposed on a steady-state depolarization. In some recordings, the typical complex spikes did not occur, presumably because the resting membrane potentials were less negative than −60 mV. The latency from the stimulus artifact to onset of CF-EPSPs was 1.9 ± 0.3 ms. After exposure of slices to 100 μM MeHg, the latency was prolonged to 2.7 ± 0.4 ms, suggesting that impulse conduction from climbing fibers to Purkinje cells was affected by MeHg. Also shown in Fig. 4 (right, data for 20 μM not shown for simplicity), at 100 μM MeHg, the amplitudes of the steady-state depolarization were almost always reduced before block of the complete climbing fiber response. Moreover, the complex spikes appeared to be more sensitive to MeHg than were the antidromically activated action potentials, because the antidromically activated action potentials remained after complex spikes were blocked completely at 30 min by 100 μM MeHg. At 20 μM MeHg, complex spikes were blocked completely at 50 min (data not shown). Initially, block of CF-EPSPs could be overcome by increasing stimulation intensity, which was similar to the effects of MeHg on PF-EPSPs. Times to complete block of CF-EPSPs by 100 μM and 20 μM MeHg were 36 ± 4 and 60 ± 8 min, respectively, which were similar to those for block of PF-EPSPs. Thus, acute administration of MeHg affects both PF-EPSPs and CF-EPSPs with similar time courses.

The resting membrane potential of Purkinje cells recorded from 24 slices before exposure to MeHg was −60 ± 4 mV under our experimental conditions. At 100 μM and 20 μM, MeHg hyperpolarized and then depolarized Purkinje cell membranes (Fig. 5). At 100 μM and 20 μM MeHg, 11 of 15 and 8 of 9 recordings showed a hyperpolarization before depolarization of Purkinje cell membranes. In most cases, decreases in amplitudes of PF-EPSPs or CF-EPSPs until complete block were usually accompanied with gradual depolarization of Purkinje cell membranes. However, injection of a hyperpolarizing current to restore membrane potentials to their original levels after PF-EPSPs and CF-EPSPs were blocked completely failed to cause recovery of either PF-EPSPs or CF-EPSPs (Fig. 5, top), suggesting that membrane depolarization caused by MeHg may contribute to its effects on PF-EPSPs and CF-EPSPs, but was not the primary cause of block of these responses.

Effects of MeHg on Somatic Action Potentials Evoked by Direct Depolarization of Purkinje Cells. One electrical property of Purkinje cells is that direct depo-
ultimately all responses were blocked by MeHg. Increases in the late half of the sustained depolarization (20 min in Fig. 6) were superimposed on bursts" (Llinás and Sugimori, 1980a) were superimposed on them. The arrow indicates the starting time of application of MeHg. Values are expressed as percentages of preMeHg exposure control. All values are the mean ± S.E. of 7 to 15 individual experiments.

Fig. 5. Time courses of effects of MeHg on resting membrane potentials of Purkinje cells in cerebellar slices. Top, traces and values (from left to right) representatively demonstrate changes in PF-EPSPs and resting membrane potentials recorded at 0, 30, and 55 min after exposure to 100 μM MeHg in a transverse slice. The asterisk indicates that after PF-EPSPs were blocked at 55 min, injecting negative current to hold the cell membrane potential at ~70 mV failed to restore PF-EPSPs. Bottom, time courses of effects of 100 μM and 20 μM MeHg on resting membrane potentials. The arrow indicates the starting time of application of MeHg. Values are expressed as percentages of preMeHg exposure control. All values are the mean ± S.E. of 7 to 15 individual experiments.

Fig. 6. Time course of effects of 100 μM MeHg on responses evoked by direct injection of depolarizing current pulse (1000 ms) through the recording electrode at Purkinje cell soma. Normally, threshold current injection caused regular repetitive firing (Na+-dependent fast somatic spikes). Note that the amplitude of individual fast somatic spikes varies due to the long digital sampling interval (1 ms). At short digital sampling intervals (0.1–0.2 ms), all spikes show similar amplitudes. The same is true for Figs. 7 and 9. In the presence of MeHg, the late half of membrane depolarization plateau became more positive and the fast somatic repetitive spikes disappeared and were replaced by low-amplitude Ca++ spike bursts with a slow rate of rise (as indicated by arrows). Each trace is a representative depiction of eight individual experiments.

lrarization of the cell soma by current injection just above the threshold level through the recording electrode usually generates regular, repetitive firing. The patterns of repetitive firing can be changed by alterations of the amplitude and duration of the injection current pulses (Llinás and Sugimori, 1980a). To determine whether MeHg acts directly on Purkinje cells to affect their intrinsic electrophysiological activity, we examined the effects of MeHg on evoked repetitive firing activity of Purkinje cells. Acute bath application of MeHg also altered the patterns of repetitive firing of Purkinje cells. Under conditions similar to those described by Llinás and Sugimori (1980a), injecting 500- to 1000-ms positive current pulses at a level slightly higher than the threshold generated a form of regular repetitive firing with a frequency of 30 to 40 spikes/s (Fig. 6). After exposure to 100 μM MeHg, the firing pattern was changed in several aspects. First, the frequency of firing or the number of so-called fast somatic spikes, described by Llinás and Sugimori (1980a), was gradually reduced. Second, during the latter half of the previously steady-state depolarization, the membrane potential became more positive and the fast somatic spikes were virtually abolished (at 5, 10, and 15 min in Fig. 6); replacing them were small amplitude, burst-like spikes (as indicated by arrows). Third, at the later stage of exposure to MeHg, oscillatory bursting activity or so-called “depolarizing spike bursts” (Llinás and Sugimori, 1980a) were superimposed on the late half of the sustained depolarization (20 min in Fig. 6). Ultimately, all responses were blocked by MeHg. Increasing current injection at this time did not restore these responses. Thus, MeHg also acted directly on Purkinje cells to suppress their electrical activity.

To test what is(are) the primary site(s) of action of MeHg in blocking PF-EPSPs or CF-EPSPs, we compared the time courses of MeHg-induced block-of-action potentials and PF-EPSPs evoked by stimulating parallel fibers with responses evoked simultaneously by directly depolarizing Purkinje cell soma through the recording electrode. At 100 μM, MeHg blocked action potentials evoked by stimulating parallel fibers at a level slightly higher than threshold within 15 min (Fig. 7, left). At this time, slightly increasing stimulus intensity could restore an action potential. Five minutes later, action potentials evoked at the increased level of stimulation were blocked again; further increasing stimulus intensity only evoked a low-amplitude and narrow-duration EPSP or local responses without an action potential superimposed on them. The residual synaptic responses were blocked eventually at 35 min. At the same time, repetitive firing of Purkinje cells evoked by depolarizing current pulses was first reduced significantly in number, then changed to burst-like firing, and finally blocked completely at 25 min (Fig. 7, right). In almost all recordings, block-of-action potentials evoked by stimulating parallel fibers and of repetitive firing evoked by current injection occurred at the same time, suggesting that they resulted from the same effect. However, complete block of the residual PF-EPSPs or local responses required a slightly longer time. Times to block by 100 μM MeHg of PF-EPSPs and repetitive firing were 33 ± 7 min and 23 ± 5 min, respectively. The same was true for effects of 20 μM MeHg on these responses. At 35 min, the repetitive firings evoked by depolarizing current pulses were blocked completely by 20 μM MeHg, whereas the residual PF-EPSPs could still be initiated at 40 min and were blocked completely only after 60-min exposure to MeHg (results not shown). We also compared time courses of block of CF-EPSPs evoked by...
stimulating climbing fibers with responses evoked simultaneously by direct depolarization of Purkinje cell soma through the recording electrode. In Fig. 8, the somatic repetitive firings were evoked by a short current pulse (50 ms). At 100 μM, MeHg blocked action potentials or complex spikes evoked by stimulating climbing fibers and the somatic repetitive firings evoked by direct depolarization of Purkinje cells through current injection with a similar time course. However, block of the residual synaptic responses evoked by stimulating climbing fibers took slightly longer (Fig. 8). In this case, complete block of the residual synaptic responses occurred at 55 min. Thus, these results suggest that the voltage-dependent responses or action potentials evoked by stimulating parallel fibers, climbing fibers, or by direct depolarization of Purkinje cells were equally sensitive to MeHg. However, the residual synaptic responses or local responses evoked by stimulating parallel or climbing fibers were slightly less sensitive to MeHg than were those voltage-dependent responses.

Effects of MeHg on Spontaneous Activity of Purkinje Cells. In addition to those unique evoked responses, Purkinje cells also displayed another well-described electrical property—spontaneous firing or autorhythmic oscillatory activity, which was observed in both extracellular and intracellular recordings.
lular recordings (Llinás and Sugimori, 1980a,b; Aubry et al., 1991; Chang et al., 1993). In hippocampal slices, spontaneous activity appeared to be less sensitive to MeHg than were evoked responses in CA1 pyramidal neurons (Yuan and Atchison, 1993). To test if this also occurred in Purkinje cells, effects of MeHg on spontaneous firing activity were examined simultaneously with those on evoked responses. Under our experimental conditions, typically within the first few minutes after penetration of their membranes, Purkinje cells displayed a mixed form of spontaneous firing, including the so-called Na⁺-dependent fast somatic repetitive action potentials, and Ca²⁺-dependent, slow dendritic spikes or bursting, as described by Llinás and Sugimori (1980a,b). After recordings were stable for 5 to 10 min, the pattern of spontaneous firing was predominantly of the repetitive, fast somatic spike form. However, after exposure to 100 μM MeHg, initially the number of fast somatic spikes was reduced and then the patterns of spontaneous firing became predominantly autorhythmic bursting activity separated by interburst hyperpolarizations at 15 to 20 min (Fig. 9, top). Subsequently, all spikes were blocked and the remaining responses were the slow rate of rise, low-amplitude oscillatory local responses. At the same time, all action potentials or repetitive spikes evoked by stimulating parallel fibers, climbing fibers, or by direct depolarization of Purkinje cell soma, and even by antidromic stimulation of Purkinje cell axons were blocked too, except for residual PF-EPSPs and CF-EPSPs. Later, all remaining responses, including the low-amplitude oscillatory local responses, the residual PF-EPSPs and CF-EPSPs, were blocked as well (Fig. 9, top). These results suggest that all somatic action potentials or repetitive spikes, whether occurring spontaneously or evoked, were similarly sensitive to MeHg. Interestingly, in many cases, after responses recorded from intracellular recordings were blocked completely upon withdrawing the recording electrode from the cells, spontaneous extracellular firing was observed. Thus, we examined further the effects of MeHg on spontaneous activity observed in extracellular recordings. Figure 9 (bottom) shows representative extracellular spontaneous firing activity. During the initial 30 min of exposure of a transverse slice to 100 μM MeHg, stimulating parallel fibers could initiate population spikes, as indicated by the arrows. After 40 min, no further evoked responses could be observed, however, spontaneous firing remained until complete block occurred at 60 min. Thus, spontaneous activity appears to be less sensitive to MeHg than are evoked responses.

**Discussion**

Previously, we demonstrated that MeHg affects both excitatory and inhibitory synaptic transmission in hippocampal slices (Yuan and Atchison, 1993,1995). However, after in vivo exposure to MeHg, the cerebellum is a more prominent target of MeHg neurotoxicity. Additionally, the circuitry of the cerebellum is quite unique, and there is clear differential cellular sensitivity to MeHg in cerebella of affected patients. Thus, we sought to examine directly the effects of acute exposure to MeHg on cerebellar synaptic transmission to determine whether cerebellar synaptic function was inherently more sensitive to MeHg as well as to characterize the effects of the metal on the two major pathways in the cerebellum. As the first step, the objective of the present study was to compare the effects of MeHg on synaptic transmission between parallel fibers or climbing fibers and Purkinje cells using extracellular and intracellular recording techniques. Acute (bath) application of 20 and 100 μM MeHg caused an initial stimulation followed by depression of synaptic transmission. This pattern is characteristic of effects of MeHg in hippocampal slices and, thus, may represent a generic pattern of effects of acute exposure of synapses to the metal. In parallel fibers, MeHg appears to block the glutamate-mediated PSRs more rapidly than it did PFVs. Moreover, across transverse and sagittal slices, MeHg affected the glutamate-mediated PSRs and CFRs with a similar time course.

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**Fig. 9.** Effects of 100 μM MeHg on spontaneous repetitive firing of Purkinje cells. Top, spontaneous repetitive firing was recorded from Purkinje cell soma in either transverse or sagittal slices using intracellular recording techniques. The asterisks indicate that at the same time, all evoked voltage-dependent responses were also blocked with the exception of the residual local responses evoked by stimulation of parallel fibers or climbing fibers. Bottom, spontaneous firing of Purkinje cells recorded from the Purkinje cell layer of a transverse cerebellar slice using extracellular recording techniques. Arrows indicate responses evoked by stimulating the parallel fibers on the surface of the molecular layer. Each trace is a representative depiction of three to five individual experiments. Calibration bars: vertical, 20 mV; horizontal, 400 ms.
cellular recordings further supported this conclusion as MeHg blocked both PF-EPSPs and CF-EPSPs with similar time courses. MeHg blocked responses evoked by direct depolarization of Purkinje cell soma with a similar time course to its effects on PF-EPSPs and CF-EPSPs. MeHg also hyperpolarized and then depolarized Purkinje cell membranes and suppressed spontaneous activity.

Purkinje cells differ from most neurons in the brain in that a single Purkinje cell receives two major excitatory synaptic inputs: an indirect input from the parallel fibers and a direct input from the climbing fibers. One Purkinje cell may make synaptic contacts with as many as 200,000 parallel fibers. On the other hand, a given Purkinje cell only makes synaptic contact with one climbing fiber; however, as many as 200 contacts may be formed between each Purkinje cell and each climbing fiber (Llinás and Walton, 1990). When activated, both synaptic responses can be easily recorded in the molecular layer of the cerebellar cortex using extracellular recording techniques, albeit using slices of different orientation. In addition, when parallel fibers are activated, action potential propagation along the parallel fibers can be recorded as the PFV by an extracellular recording electrode in the molecular layer. As expected, exposure of cerebellar slices to MeHg caused stimulation and then suppression to complete block of these field potentials. In hippocampal slices, MeHg caused similar effects on population spikes recorded from CA1 pyramidal neurons. The time course of effects of MeHg on the two different slice preparations is quite similar. For example, at 20 µM, MeHg blocks the EPSP generated in CA1 neurons at 63 ± 6 min (Yuan and Atchison, 1995) and in the cerebellar slice at 57 ± 6 min. The early stimulatory effects of MeHg on hippocampal CA1 excitatory synaptic transmission are apparently due primarily to a preferential action of MeHg on type A γ-aminobutyric acid receptor-mediated inhibitory synaptic transmission leading to disinhibition of excitatory synaptic function (Yuan and Atchison, 1997). The same mechanism may apply to the stimulatory effects of MeHg on the glutamate-mediated postsynaptic responses in Purkinje cells such as PSRs evoked by stimulating parallel fibers and CFRs evoked by stimulating climbing fibers. Purkinje cells also receive inhibitory inputs directly from two types of GABAergic interneurons, the stellate and basket cells, and indirectly from Golgi cells (Llinás and Walton, 1990; Fig. 1). Golgi cells, which are excited by mossy, climbing, and parallel fibers, exert an inhibitory action on granule cells and indirectly modulate activity of Purkinje cells. It is possible that this disinhibition in response to MeHg is also responsible for the early increase in PFV amplitude. The unexpected result is that MeHg blocked the presynaptic fiber response apparently more slowly than it blocked the glutamate-mediated PSRs and CFRs. Pathologically, the granule cells are well known to be highly sensitive to MeHg (Hunter and Russell, 1954; Takeuchi et al., 1962; Chang, 1977; Syversen, 1981) and their axons, which form the parallel fibers, are unmyelinated and injured by MeHg during chronic exposure. Thus, we sought to determine whether, during acute exposure to MeHg, initiation and propagation of action potentials along parallel fibers would be affected by MeHg as readily as are the postsynaptic responses. Results suggest that the synaptically mediated PSRs are more sensitive than conduction along the parallel fibers. The relative lack of sensitivity of PFVs to MeHg compared with PSRs and CFRs suggests that, at least acutely, the granule cell axons are no more sensitive to MeHg than are other cerebellar neuronal processes, and like peripheral synapses, the process of synaptic transmission is more sensitive to block by MeHg than is the process of impulse conduction (Juang, 1976; Atchison and Narahashi, 1982). Perhaps, this is in part due to the relatively high Na+ channel density in the presynaptic parallel fibers, making them more resistant to MeHg, or perhaps it simply reflects a preferential effect of MeHg on either the glutamate release processes from parallel fibers and climbing fibers or postsynaptic glutamate receptor functions.

To determine how MeHg caused its effects on the field potentials, PF-EPSPs and CF-EPSPs were examined using intracellular recording techniques. Consistent with the results obtained from extracellular recordings, MeHg blocked both PF-EPSPs and CF-EPSPs with similar time courses, even though the two responses were generated from different synaptic pathways with distinct electrophysiological characteristics. The PF-EPSPs, or single peaked and graded responses, are simple spikes. Each parallel fiber, when activated, produces a small synaptic current that must sum at the initial segment of the Purkinje cell to produce an action potential. Thus, activation of parallel fibers leads to generation of voltage- and Na+-dependent simple spikes graded as a function of the summation of synaptic currents from many parallel fiber synapses. Conversely, the CF-EPSPs generated by stimulation of climbing fibers are all or none Ca²⁺-dependent responses (Crepel and Delhaye-Bouchaud, 1978; Llinás and Sugimori, 1980a,b; Crepel et al., 1981, 1982; Llinás and Walton, 1990; Llano et al., 1991; Stuart and Häusser, 1994). Stimulation of climbing fibers produces a large desynchronized depolarization of Purkinje cell dendrites, which then activates the dendritic Ca²⁺ channels to initiate the slow dendritic Ca²⁺ spikes. It is not surprising that MeHg blocked both responses as it blocks currents carried through both Na⁺ channels (Shrivastav et al., 1976; Quandt et al., 1982; Shafer and Atchison, 1992; Leonhardt et al., 1996) and Ca²⁺ channels (Shafer and Atchison, 1989; Shafer et al., 1990; Shafer and Atchison, 1991, 1992; Sirois and Atchison, 1996; Leonhardt et al., 1996). The similarity in blocking both responses suggests that MeHg may act via a similar mechanism to block both PF-EPSPs and CF-EPSPs. In CF-EPSP recordings, on the other hand, the early suppression of the steady-state depolarization and block of complex spikes almost always occurred before block of the antidromically activated spikes, suggesting that the orthodromically activated synaptic responses were more sensitive to MeHg than were antidromically activated responses. Inasmuch as the antidromically activated responses bypass synaptic transmission, this implies that actions of MeHg on synaptic transmission underlie this differential sensitivity. Unlike the effects of MeHg on EPSPs recorded from the CA1 region of hippocampal slices, MeHg did not cause a consistent, significant early stimulatory effect on PF-EPSPs and CF-EPSPs, although in some slices, it caused a slight and transient early increase. These results were also inconsistent with those obtained from extracellular recordings, in which MeHg caused a significant early increase in amplitude before suppression of the field potentials. The difference between results obtained from extracellular and intracellular recordings may indicate that MeHg does not significantly affect the response of an individual Purkinje cell to a given stimulus, but rather induces...
recruitment of additional Purkinje cells which fire synchronously at the early stage of exposure to MeHg.

To determine whether MeHg acts on Purkinje cells to block PF-EPSPs or CF-EPSPs, effects of MeHg on responses evoked by direct depolarization of Purkinje cells with current injection at the soma were examined and compared with those of MeHg on PF-EPSPs and CF-EPSPs. Normally, injection of a short (50–100 ms) or long current pulse (500–1300 ms) at the threshold level in Purkinje cell soma generates regular, repetitive firing, fast somatic spikes. As the amplitude of the current injection pulse is increased, particularly for long current pulses, the typical repetitive firing is replaced, near the end of the pulse, by a complex, low-amplitude depolarizing spike burst (Llinàs and Sugimori, 1980a). The fast somatic spike is a low-threshold, voltage- and Na⁺-dependent response, which is blocked by removal of extracellular Na⁺ or by application of tetrodotoxin (TTX). The depolarizing spike burst, on the other hand, is a slow rate of rise, high-threshold, voltage- and Ca²⁺-dependent response, which is TTX-insensitive and blocked by removal of extracellular Ca²⁺ or by application of Ca²⁺ channel blockers such as Co²⁺, Cd²⁺, or Mn²⁺ (Llinàs and Sugimori, 1980a,b; Aubry et al., 1991; Chang et al., 1993). In the presence of MeHg, and without changing the amplitude of threshold current injection, the patterns of the repetitive firing induced were altered such that they resembled responses caused by increasing current pulses under normal conditions. Thus, MeHg appears to alter Purkinje cell-membrane ion conductances in the same way that they are altered by increasing stimulus intensity. Normally, generation of this Ca²⁺-dependent depolarizing spike burst requires higher stimulus intensity because of its high threshold nature. However, exposure of slices to MeHg induced an identical response even at normal threshold stimulus level. That suggests that MeHg may initially change the threshold level for activation of Ca²⁺ channels. Perhaps this effect occurs as a result of loss of feedback type of inhibition at the level of basket or stellate cell onto the Purkinje neuron. Ultimately, as it did with CA1 pyramidal cells in hippocampal slices, MeHg blocked all voltage-dependent responses including both Na⁺- and Ca²⁺- dependent spikes evoked by stimulation of parallel or climbing fibers or by direct current injection at Purkinje cell somata with a similar time course. This suggests that MeHg primarily acts at the Purkinje cells via similar mechanisms to block these voltage-dependent responses. A slightly longer time was required for complete block by MeHg of the synchronically activated local responses or the residual PF-EPSPs or CF-EPSPs compared with those required for blocking voltage-dependent responses. However, only a slight reduction in amplitude of the EPSP would be sufficient to block generation of the postsynaptic spike, while leaving a subthreshold EPSP. Thus, glutamate receptor function may also be affected by MeHg, although perhaps relatively less sensitively than that of voltage-dependent channels. This is consistent with the results that in the hippocampus, MeHg blocked orthodromically activated synaptic responses more rapidly than it did antidromically activated responses (Yuan and Atchison, 1993, 1994). In addition, the prolonged latencies from stimulus artifact to onset of PF-EPSPs and CF-EPSPs suggest that current conduction from parallel fibers or climbing fibers to the dendrites of Purkinje cells were affected by MeHg. Thus, once again, MeHg apparently acts at multiple sites to block synaptic transmission between parallel fibers or climbing fibers and Purkinje cells.

In addition to effects of MeHg on the evoked repetitive firing of Purkinje cells, MeHg also changed the patterns of spontaneous, repetitive firing of Purkinje cells. Normally, spontaneous repetitive firing consists of predominantly Na⁺-dependent, fast somatic spikes and a low-amplitude, Ca²⁺-dependent, slow dendritic spike burst (Llinàs and Sugimori, 1980a). After exposure to MeHg, the patterns of spontaneous firing initially changed to one of predominantly Ca²⁺-dependent autorhythmic bursts. This effect of MeHg is very similar to that of TTX on Purkinje cell spontaneous firing (Aubry et al., 1991; Chang et al., 1993). Application of TTX to cerebellar slices suppressed Na⁺ spikes of Purkinje cells and induced Ca²⁺-dependent oscillatory firing activity. This oscillatory firing activity was thought to be maintained by an intrinsic property of Purkinje cells because it remained after block of both excitatory and inhibitory synaptic inputs to Purkinje cells (Chang et al., 1993). The proposed mechanism responsible for TTX-induced oscillatory activity of Purkinje cells was that block of Na⁺ channels leads to activation of Na⁺/Ca²⁺ exchanger with a net gain of intracellular Ca²⁺ (Aubry et al., 1991; Chang et al., 1993). Compared with TTX, MeHg blocks both Na⁺ and Ca²⁺ channels and increases intracellular concentration of Ca²⁺ ([(Ca²⁺)i]) in a variety of cells including primary cultures of cerebellar granule cells (Hare et al., 1993; Hare and Atchison, 1995a,b; Marty and Atchison, 1997). Thus, the MeHg-induced oscillatory burst activity may be related to an effect on regulation of [Ca²⁺]i, of Purkinje cells. Perhaps, initially, MeHg blocks Na⁺ channels as TTX does to activate the Na⁺/Ca²⁺ exchanger (Aubry et al., 1991; Chang et al., 1993), which unmasks intrinsic oscillatory activity of Purkinje cells by an unknown mechanism, increases [Ca²⁺]i, and depolarizes Purkinje cell membranes to generate the slow Ca²⁺ spikes. Subsequently, the increase in [Ca²⁺]i, might activate Ca²⁺-dependent K⁺ channels, leading to hyperpolarization of Purkinje cells to return their membrane potentials toward the resting level. As the K⁺ channels close, another cycle begins. However, at the late stage of exposure of slices to MeHg, all action potentials or voltage-dependent responses were blocked and what was left were only those low-amplitude oscillatory local responses. These local oscillatory responses were later blocked completely along with the synchronically activated local responses (residual PF-EPSPs and CF-EPSPs) at the same time, suggesting that the Ca²⁺-dependent oscillatory burst activity of Purkinje cells is of dendritic origin. This is consistent with the conclusion of Llinàs and Sugimori (1980a,b). Spontaneous firing is also observed in extracellular recordings. Interestingly, MeHg blocked the spontaneous responses more slowly than it did the evoked responses. In addition, in many cases, after evoked responses recorded by intracellular recording were blocked, the extracellular spontaneous responses remained, suggesting that they were less sensitive to MeHg than were evoked responses. This is consistent with the effects of MeHg on neuromuscular transmission (Atchison and Narahashi, 1982) in which spontaneous release of acetylcholine remained observable at the time evoked release of acetylcholine was blocked completely by MeHg. Thus, mechanisms responsible for block by MeHg of spontaneous and evoked responses differ. Moreover, it appears that MeHg takes a longer time to block extracellular responses than it
does intracellular responses. This may be due simply to the fact that in intracellular recording, electrode penetration of the cell causes injury of the membrane, which accentuates the action of MeHg. Alternatively, accessibility of MeHg to cells located on the surface and in the deep tissue of slices differs. The extracellular recording electrode picks up firing from a population of cells that may include those located at both the surface and deep tissues. For those cells in the deep tissue, it will take a longer time for MeHg to accumulate there and, hence, a longer time to block their responses.

In conclusion, MeHg caused biphasic effects on synaptic transmission between parallel fibers or climbing fibers and Purkinje cells in cerebellar slices. MeHg appears to act primarily at the postsynaptic Purkinje cells to cause these effects because it blocked responses evoked by directly depolarizing Purkinje cells. However, multiple actions, including hyperpolarizing and depolarizing the Purkinje cell membranes, blocking current conduction, and affecting glutamate receptor functions may also be involved in these effects. MeHg blocked spontaneous firing of Purkinje cells and acted in a manner similar to TTX to induce a Ca$^{2+}$-dependent, spontaneous oscillatory burst activity in Purkinje cells. This is superficially consistent with the findings that MeHg increases [Ca$^{2+}$]i, in a variety of cell types. In general, the effects of MeHg on electroresponsiveness of Purkinje cells in cerebellar slices are similar to those on CA1 pyramidal cells in hippocampal slices. Thus, at least for acute exposure, the enhanced sensitivity of the cerebellum to MeHg toxicity cannot be ascribed to a simple increased sensitivity of cerebellar circuitry to the metal.

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Send reprint requests to: Address all correspondence including reprint requests to: Dr. William D. Atchison, Ph.D., Michigan State University, Department of Pharmacology and Toxicology, B-331 Life Sciences Building, East Lansing, Michigan 48824-1317. Email: atchison@pilot.msu.edu