Action of Methylmercury on GABA_\textsubscript{A} Receptor-Mediated Inhibitory Synaptic Transmission Is Primarily Responsible for Its Early Stimulatory Effects on Hippocampal CA1 Excitatory Synaptic Transmission

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ABSTRACT

Bath application of methylmercury (MeHg) causes an early stimulation before block of synaptic transmission in the CA1 region of hippocampal slices. Effects of MeHg and Hg\textsuperscript{2+} on inhibitory postsynaptic potentials (IPSPs) or currents (IPSCs) and excitatory postsynaptic potentials (EPSPs) or currents (EPSCs) were compared to test whether or not early block by MeHg of GABA\textsubscript{A}-mediated inhibitory synaptic transmission and MeHg-induced alterations of the resting membrane potentials of CA1 neurons contribute to this initial enhancement of excitability. MeHg affected IPSPs and IPSCs similarly, and more rapidly than EPSPs and EPSCs. In contrast, although Hg\textsuperscript{2+} blocked IPSPs more rapidly than EPSPs, times to block of IPSCs and EPSCs by Hg\textsuperscript{2+} were virtually identical when CA1 neurons were voltage-clamped at their resting membrane potential levels. MeHg increased EPSC amplitudes before their subsequent decrease even when CA1 neuronal membranes were voltage-clamped at their resting potentials. This suggests that effects of MeHg on CA1 cell membrane potentials are not a major factor for MeHg-induced early stimulation of hippocampal synaptic transmission. Effects of MeHg and Hg\textsuperscript{2+} on the reversal potentials for IPSCs also differed. Both metals blocked all outward and inward currents generated at different holding potentials. However, MeHg shifted the current-voltage (I/V) relationship to more positive potentials, although Hg\textsuperscript{2+} shifted the I/V curve to more negative potentials. Hg\textsuperscript{2+} was a less potent blocker of IPSCs and EPSPs or EPSCs than was MeHg. To determine if the early increase in amplitude of population spikes or EPSPs is due to an action of MeHg at GABA\textsubscript{A} receptors, extracellular recordings of population spikes and intracellular recordings of EPSPs were compared with or without pretreatment of hippocampal slices with bicuculline. After preincubation of slices with 10 \mu M bicuculline for 30 to 60 min, MeHg only decreased the amplitudes of population spikes and EPSPs to block; no early increase of synaptic transmission occurred. Pretreatment of slices with strychnine, did not prevent MeHg-induced early increase in population spikes. MeHg also blocked responses evoked by bath application of muscimol, a GABA\textsubscript{A} agonist. Thus, block by MeHg of GABA\textsubscript{A} receptor-mediated inhibitory synaptic transmission may result in disinhibition of excitatory hippocampal synaptic transmission, and appears to be primarily responsible for the initial excitatory effect of MeHg on hippocampal synaptic transmission.

Acute bath application to hippocampal slices of the neuronal metal MeHg causes a concentration- and time-dependent biphasic effect on synaptic transmission in the CA1 region. Initially MeHg increases the amplitudes of field potentials recorded extracellularly (Yuan and Atchison, 1993, 1994) and EPSPs recorded intracellularly before suppression of them to block (Yuan and Atchison, 1995a). MeHg also blocks the recurrent IPSPs (Andersen et al., 1964a,b) in the CA1 region. IPSPs appeared to be more sensitive to MeHg than EPSPs, because block of IPSPs occurred earlier than did block of EPSPs. The time to the early suppression of IPSP amplitudes appeared to correspond to the onset of the early increase in amplitudes of both population spikes and EPSPs. This suggests that the reduced IPSPs contribute to the early increase in amplitudes of population spikes and EPSPs.

ABBREVIATIONS: MeHg, methylmercury; GABA, \gamma-aminobutyric acid; EPSPs, excitatory postsynaptic potentials; EPSCs, excitatory postsynaptic currents; IPSPs, inhibitory postsynaptic potentials; IPSCs, inhibitory postsynaptic currents; ACSF, artificial cerebrospinal fluid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; AP-5, amino-5-phosphono pentanoic acid; I/V curve, voltage-current relationship; DMSO, dimethyl sulfoxide.
MeHg suppresses the GABA-induced chloride current in dorsal root ganglion cells (Arakawa et al., 1991) and modulates the muscimol-induced increases in the \([3H]\)flunitrazepam binding to GABA\(_A\) receptors in washed cerebellar membranes (Komulainen et al., 1995). Thus, we hypothesized that block by MeHg of GABA\(_A\) receptor-mediated inhibitory synaptic transmission results in disinhibition of hippocampal excitatory synaptic transmission, and is at least partly responsible for the initial stimulatory effects of MeHg on CA1 hippocampal synaptic transmission. However, MeHg also caused biphasic changes in resting membrane potentials, i.e., initial hyperpolarization and then depolarization of pyramidal CA1 neurons in hippocampal slices (Yuan and Atchison, 1995a) and rat forebrain synaptosomes (Hare and Atchison, 1992). This effect alone could influence the observed changes in IPSP and EPSP amplitudes. Thus, nonspecific effects of MeHg on resting membrane potentials may also be involved in its early effects on synaptic transmission.

To test this hypothesis, extracellular recordings of population spikes, intracellular recordings of EPSPs and IPSPs and single-microelectrode voltage-clamp recordings of EPSCs and IPSCs from CA1 pyramidal neurons were compared with or without pretreatment of hippocampal slices with bicuculline, a GABA\(_A\) antagonist. We sought to determine: 1) whether or not MeHg and inorganic mercury (Hg\(^{11+}\)) affect IPSPs and EPSPs in hippocampal CA1 neurons differentially, because they differentially affect GABA-mediated chloride currents in dorsal root ganglion neurons (Arakawa et al., 1991; Huang and Narahashi, 1996) and field potentials recorded in CA1 neurons of hippocampal slices (Yuan and Atchison, 1994); 2) whether or not the differential block by MeHg of IPSPs and EPSPs is due to nonspecific effects of MeHg on resting membrane potentials and 3) whether or not block of GABA\(_A\)-mediated IPSPs is primarily responsible for the early stimulation of hippocampal synaptic transmission. Because this early stimulation is a characteristic of the effects of MeHg induced on central synaptic transmission, we sought to understand how this effect occurs, and how it pertains to the overall process of MeHg-induced neurotoxicity.

**Materials and Methods**

**Materials.** Methylmercuric chloride, purchased from ICN Bio-medical, Inc. (Costa, CA), was dissolved in deionized water to a final concentration of 5 mM to serve as stock solution. The applied solutions (4–500 \(\mu\)M) were diluted with ACSF with the following composition (in mM): NaCl 124; KCl 5; MgSO\(_4\) 2; KH\(_2\)PO\(_4\) 1.25; NaHCO\(_3\) 26; CaCl\(_2\) 2 and d-glucose 10; pH was set at 7.4 just before superfusion. MeHg and other chemicals were applied acutely to slices by bath application at a rate of 1.2 to 1.5 m\(\mu\)l/min with a Gilson (Middleton, WI) infusion pump. Strychnine hydrochloride and muscimol were purchased from Sigma Chemical Co. (St. Louis, MO). Muscimol (25–100 \(\mu\)M) was applied to slices for 15 to 30 sec at an interval of 10 min to avoid desensitization of GABA\(_A\) receptors. CNQX, DNQX, AP-5 and (-)-bicuculline methobromide were obtained from Research Biochemical International (Natick, MA). CNQX or DNQX were dissolved first in DMSO and then diluted further with ACSF. The final concentration of DMSO in the applied solution was less than 0.02% (v/v), which has no significant effects on synaptic transmission.

**Preparation of hippocampal slices.** Hippocampal slices were prepared as described previously (Yuan and Atchison, 1993). Briefly, hippocampi isolated from brains of male Sprague-Dawley rats (125–150 g, Harlan Industries, Madison, WI) were sectioned transversely at 4°C to slices of approximately 400-\(\mu\)m thickness. Slices were transferred immediately to a recording chamber and incubated for at least 60 min before electrophysiological recording. A humidified gas mixture of 95% O\(_2\)/5% CO\(_2\) was circulated over the slices continuously by bubbling in the bath water. During recording, two or three slices were kept in the chamber at a given time. The rest were maintained in a reservoir chamber for later use. All experiments were conducted at 33 to 35°C. All experiments were replicated at least four times, and no more than one slice from a given rat was used for a particular experiment.

**Electrophysiological procedures.** Conventional extracellular and intracellular recordings were made in the CA1 region of the hippocampal slice. Monopolar tungsten electrodes (3 M\(\Omega\), FHC, Brunswick, ME) were used as stimulation electrodes. Borosilicated glass microelectrodes (o.d. 1.0 mm; i.d. 0.5 mm, WPI, Inc., New Haven, CT) filled with ACSF (5–15 M\(\Omega\)) or 3 M potassium acetate (80–120 M\(\Omega\)) were used for extracellular or intracellular recording, respectively. Population spikes were evoked by orthodromic-stimulation of Schaffer collaterals at an intensity level (usually 2–4 V) that gives a population spike amplitude approximately 50% of the maximum amplitude as evoked by maximum stimulation. Intracellular EPSPs were recorded at CA1 cell soma by subthreshold stimulation (0.2 Hz) of Schaffer collaterals; typically a 0.1 to 0.2 nA negative D.C. current was applied through the recording electrode to maintain the cell membrane in a somewhat hyperpolarized state to avoid evoking action potentials. The recurrent IPSPs (Andersen et al., 1964a, 1964b) were recorded by subthreshold stimulation of the alveus. IPSCs and EPSCs were recorded using single-microelectrode voltage clamp techniques (Johnston et al., 1980; Johnston and Brown, 1981, 1984). The sample frequency was set at 8 kHz or as high as possible. When measuring the current-voltage relationship, voltage step commands were generated from an internal step command generator and manually controlled by the thumbwheel switch on the front panel of an Axoclamp-2 amplifier. For each voltage step, the cell was held at that potential for 30 to 40 sec to obtain at least three to five traces of IPSCs. The membrane input resistance was monitored by D.C. current injection through the recording electrode. All stimulus pulses were generated from a Grass S88 stimulator (Grass, Inc., Quincy, MA) at 0.2 Hz and 0.1-msec duration and isolated with a Grass SIU5 stimulus isolation unit (Grass, Inc.). Recorded signals were amplified (Axoclamp-2, Axon Instruments Inc., Foster City, CA), displayed on a 2099-D digital oscilloscope (Nicolet Instruments, Verona, WI) and recorded simultaneously to both floppy disks and magnetic tape by using a FM instrumentation recorder (model B, Vetter Instruments, Rebersburg, PA) for later analysis. All measurements in this reported paper were made based on the peak amplitude of response.

**Statistical analysis.** Data were collected continuously before and during application of MeHg and analyzed statistically using Student’s \(t\) test or paired \(t\) test or a one-way analysis of variance; Dunnett’s procedure was used for post hoc comparisons (Steel and Torrie, 1980). Values were considered statistically significant at \(P < .05\).

**Results**

**Comparative effects of MeHg and Hg\(^{2+}\) on IPSPs and EPSPs or IPSCs and EPSCs.** As shown in our previous report (Yuan and Atchison, 1995a), 100 \(\mu\)M MeHg blocked IPSPs more rapidly than it did EPSPs; times to block were 25 ± 2 and 45 ± 3 min, respectively (fig. 1, top). In some slices, both EPSPs and IPSPs were recorded simultaneously in the same neuron. In these recordings, an early increase in EPSP amplitude or even firing of action potentials often accompanied the decrease in IPSP amplitude at the early times of application of MeHg. At the same concentration, Hg\(^{2+}\) blocked IPSPs with a time course similar to that of
MeHg. However, Hg\textsuperscript{11} blocked EPSPs (63 ± 10 min) even more slowly than did MeHg. Both MeHg and Hg\textsuperscript{11} alter resting membrane potentials of various excitable cells (Juang and Yonemura, 1975; Juang, 1976; Shrivastav et al., 1989; Miyamoto, 1983; Kauppinen et al., 1989; Hare and Atchison, 1992). In hippocampal slices, acute bath application of MeHg or Hg\textsuperscript{++} depolarized the CA1 neuronal membrane. However, in many slices hyperpolarization occurred before depolarization (Yuan and Atchison, 1995a, 1995b). Because amplitudes of both IPSPs and EPSPs are affected by the membrane potential driving force, polarizing the cell membrane could contribute indirectly to effects of MeHg or Hg\textsuperscript{++} on synaptic potential amplitude. As such, single-microelectrode voltage-clamp was used to examine the effects of mercurials on the IPSCs and EPSCs, and thus determine whether nonspecific effects of MeHg or Hg\textsuperscript{++} on CA1 pyramidal cell resting potential contributed to the observed changes in EPSP and IPSP amplitude. IPSCs were recorded after pretreatment of slices for 30 min with and in the continuous presence of 20 μM AP-5 and 10 μM CNQX or DNQX in ACSF to block NMDA receptor- and non-NMDA receptor-mediated excitatory synaptic transmission. When CA1 neuronal membrane potentials were held at their resting levels (−67 ± 2 mV), times to block of IPSCs and EPSCs for MeHg (100 μM) were virtually identical to those for block of IPSPs and EPSPs (fig. 1, bottom). Hg\textsuperscript{++} (100 μM) blocked EPSCs with a time course similar to that on EPSPs; however, it blocked IPSCs more slowly than it did IPSPs. Times to block of IPSCs and EPSCs by Hg\textsuperscript{++} were 69 ± 12 and 65 ± 12 min, respectively. Moreover, MeHg still caused an early increase in EPSC amplitude prior to suppressing it even under voltage-clamp conditions (fig. 2). Thus changes in resting membrane potentials are not a primary factor for effects of MeHg on IPSPs and EPSPs or effects of Hg\textsuperscript{++} on EPSPs. Effects of Hg\textsuperscript{++} on IPSPs, however, may be due in part to alterations of resting membrane potential.

**Comparative effects of MeHg and Hg\textsuperscript{++} on current-voltage relationship of IPSCs.** Figures 3 compares the effects of 100 μM MeHg and Hg\textsuperscript{++} on a family of IPSCs evoked at potentials of −40 to −90 mV. Figure 4 depicts the current-voltage relationship (I/V curve) for these IPSCs. The IPSC reversal potential is approximately −75 mV in the absence of MeHg which is close to the equilibrium potential for Cl\textsuperscript{−} as predicted by the Nernst equation, and similar to these values obtained by Benardo (1993) and Pitler and Alger (1994), indicating that these IPSCs are primarily GABA\textsubscript{A}.
receptor-mediated chloride currents. MeHg suppressed both outward and inward currents, this effect usually started after 5 min of application of 100 μM MeHg. As shown in Figures 3 and 4 (left) exposure of slices to 100 μM MeHg for 15 min, resulted in depression of all IPSCs evoked at holding potentials of −40 to −90 mV. The I/V curve and the reversal potential were shifted to more positive potentials. In contrast, whereas 100 μM Hg^{2+} suppressed both outward and inward currents, Hg^{2+} initially caused an increase in the outward current prior to suppressing it. Moreover, Hg^{2+} shifted the I/V curve and the reversal potential to a more negative potential direction (fig. 4). At 20 μM the respective effects of MeHg or Hg^{2+} were similar but the latency to onset of action was much longer than at 100 μM (results not shown).

Comparative effects of MeHg and bicuculline on population spikes. Because MeHg suppresses the GABAA receptor-mediated chloride currents, we sought to determine if its effects are similar to those of bicuculline, a selective GABAA receptor antagonist. To test this, we compared the effects of 20 to 500 μM MeHg and 10 μM bicuculline on population spikes. We used these higher concentrations of MeHg because we previously showed that the higher concentrations of MeHg induced a more rapid and noticeable increase in population spikes which was often accompanied by repetitive firing (Yuan and Atchison, 1993). At 20 to 500 μM, MeHg caused a concentration- and time-dependent early increase in amplitudes of population spikes prior to blocking them (fig. 5). Higher concentrations (100 and 500 μM) of MeHg induced repetitive firing in response to single shock stimuli, suggesting that membrane excitability was increased. The early stimulatory effects of MeHg on population spikes were similar to those of bicuculline on population spikes.

Effects of bicuculline pretreatment on MeHg-induced early stimulation of synaptic transmission. The early stimulation of excitatory synaptic transmission may be due primarily to MeHg-induced suppression of GABAA receptor-mediated chloride currents. This in turn may lessen the inhibitory effects of interneurons on excitatory synaptic transmission. If so, then pretreatment of slices with bicuculline to block GABAA receptor-mediated chloride currents should eliminate or suppress the MeHg-induced early increases in population spike amplitudes. To test this, we compared effects of 20 and 100 μM MeHg on population spike amplitudes in the presence or absence of bicuculline. Incubation of slices with 10 μM bicuculline for 5 to 10 min increased the amplitudes of population spikes significantly; moreover the single spike response gradually changed to multiple spike responses. After 30 to 60 min of bicuculline, population spike amplitudes typically increased to and stabilized at 150 to 200% of control. At this point, two sets of experiments were designed to examine the effects of bicuculline on the early stimulation by MeHg of excitatory synaptic transmission. In the first set of experiments, 20 or 100 μM MeHg plus 10 μM bicuculline were added to the ACSF with no change in stimulus intensity. Under these conditions, MeHg still suppressed population spike amplitude as did MeHg alone, but in four of six slices caused no further significant early increase in population spike amplitudes (fig. 6, left). The second set of experiments was performed under reduced stimulus intensity. The reason for doing this was that we were concerned that pretreatment of slices with bicuculline might increase population spike amplitudes to a ceiling amplitude, above which MeHg was unable to cause further increase, thus masking the actual effect of MeHg on population spikes. Thus, the stimulus intensity was reduced to a level that gave population spike amplitudes approximately equal to the control level before bicuculline treatment, after the bicuculline-induced increase had stabilized. MeHg (20 or 100 μM) plus 10 μM bicuculline were then applied to the slices. As seen with the results of the first set of experiments, MeHg did not cause any statistically significant early increase in popula-

![Fig. 3. Comparison of effects of 100 μM MeHg (A) or Hg^{2+} (B) on IPSCs recorded at different holding potentials. IPSCs were evoked by presynaptic stimulation of Schaffer collaterals and recorded at CA1 neuronal soma of hippocampal slices in the presence of 20 μM AP-5 and 10 μM DNBQX. Time 0 min represents IPSCs recorded at different holding potentials before application of MeHg or Hg^{2+}. Time 15 or 25 min indicates the time after beginning perfusion of the slice with 100 μM MeHg or Hg^{2+}. Calibration bars: vertical, 300 pA; horizontal, 50 msec.](image-url)
tion spike amplitudes but reduced or blocked completely population spikes in three of four and five of seven slices at 20 and 100 \( \mu M \) MeHg, respectively (fig. 6, left). In the remaining slices there was a 10 to 15% early increase in population spike amplitudes before block by MeHg. This effect was not significant, and is masked in Figure 6 due to averaging of the time courses from the individual experiments. Without pretreatment of slices with bicuculline, 20 and 100 \( \mu M \) MeHg caused the typical biphasic changes in amplitudes of population spikes, although the early increase in amplitude induced by 20 \( \mu M \) MeHg was not as prominent as that caused by 100 \( \mu M \) MeHg (fig. 6). Due to variations in time course of effects of MeHg among the individual experiments, figure 6 does not show any decrease in population spike amplitudes after exposure to 20 \( \mu M \) MeHg alone for 120 min. However, prolonging exposure of slices to 20 \( \mu M \) MeHg to 150 to 180 min, caused block of all population spikes (results not shown). It appears that MeHg blocked responses more rapidly in slices treated with bicuculline than in slices not pretreated with bicuculline. To test if bicuculline would prevent early increases in EPSP amplitude induced MeHg, effects of MeHg on EPSPs were examined in the presence of 10 \( \mu M \) bicuculline as was seen in Figure 6 for field potential recordings. Thus the early stimulatory effects of MeHg on hippocampal transmission appear to be related to its actions on GABA\(_A\) receptors.

The results of the previous experiment do not rule out the possibility that MeHg directly affects GABA release from interneurons via a presynaptic mechanism. Thus, to test if the effects of MeHg are due to a direct action on GABA\(_A\) receptors we examined the effects of MeHg on responses evoked by muscimol, a GABA\(_A\) agonist. Bath application of 25 to 100 \( \mu M \) muscimol to slices for 15 to 30 sec caused a concentration-dependent depolarization of CA1 pyramidal neurons. It usually took about 6 to 10 min of wash to restore the depolarized membrane back to the premuscimol application baseline. The muscimol-evoked responses were blocked rapidly by 20 \( \mu M \) bicuculline (fig. 8, top), suggesting that they are GABA\(_A\) receptor-mediated responses. MeHg also blocked these muscimol-evoked responses (fig. 8, bottom), which was consistent with the report of Komulainen et al. (1995). Times to block by 100 \( \mu M \) MeHg of muscimol-evoked depolarization varied from 20 to 50 min in 11 experiments, depending on the concentration and duration of muscimol application. MeHg blocked the depolarization evoked by 25 \( \mu M \) muscimol more rapidly than that evoked by 50 or 100 \( \mu M \) muscimol. In these experiments, it is difficult to determine the exact time to block by MeHg of muscimol-evoked responses due to the long time interval required for washing out muscimol from slices before the next application. However, the times to block of muscimol-evoked responses, especially those evoked by lower concentrations of muscimol, were generally similar to those to block of IPSPs or IPSCs by MeHg. This suggests a direct action of MeHg at the GABA\(_A\) receptor sites although additional presynaptic mechanisms could still occur.

**Effects of strychnine on MeHg-induced early stimulation of CA1 synaptic transmission.** GABA is generally
believed to be the major inhibitory transmitter in the mammalian central nervous system. However, glycine also serves as an inhibitory transmitter in the central nervous system, especially in the spinal cord and brain stem (Aprison and Daly, 1978; Pycock and Kerwin, 1981; McCormick, 1990). Additionally, glycine can potentiate the action of glutamate at NMDA receptors (Johnson and Ascher, 1987), although this response is generally assumed to be strychnine insensitive (Kishimoto et al., 1981). To test whether or not a putative glycine receptor also plays a role in MeHg-induced early stimulatory effects on hippocampal synaptic transmission, slices were perfused with 50 μM strychnine, a glycine receptor antagonist, before and during exposure to 20 or 100 μM MeHg. In a similar manner to that of bicuculline, strychnine also caused a significant increase in population spike amplitudes and induced repetitive firing, although not as prominently as did bicuculline. However, unlike the effects of MeHg on population spikes in the presence of bicuculline, MeHg caused a further significant increase in population spike amplitude above that already elevated by strychnine. This effect occurred irrespective of whether or not stimulus intensity was reduced (fig. 6). Thus glycine receptors do not play a major role involved in the MeHg-induced early stimulation of hippocampal synaptic transmission. Figure 9 summarizes the effects of MeHg, bicuculline and strychnine alone and in combination with MeHg on population spike amplitude. Clearly, MeHg, bicuculline and strychnine all increase population spike amplitudes significantly. However, pre-treatment of slices with bicuculline prevented the MeHg-induced early increase in population spike amplitudes, whereas pre-treatment of slices with strychnine failed to do so.

Discussion

Previously we showed that acute bath application of MeHg caused an initial stimulation of hippocampal synaptic transmission prior to suppression to block (Yuan and Atchison, 1993, 1995a). Under similar conditions, Hg2+ blocked synaptic transmission in the CA1 region of hippocampal slices but did not induce the early stimulatory effects (Yuan and Atchison, 1994). The primary objective of the present study was to identify the potential factor(s) responsible for the early stimulatory effects of MeHg on hippocampal synaptic transmission. Previous results of microelectrode current-clamp recordings suggested that effects of MeHg on inhibitory synaptic transmission and on resting membrane potentials may be involved in the MeHg-induced early stimulation of hippocampal synaptic transmission, because MeHg blocked IPSPs more rapidly than it did EPSPs, and caused biphasic changes in resting membrane potentials of CA1 pyramidal neurons (Yuan and Atchison, 1995a). In our study, we reconfirmed that IPSPs are more sensitive to MeHg than are EPSPs, and demonstrated that this effect is not related to MeHg-induced changes in resting membrane potentials of CA1 neurons, because times to block by MeHg of IPSCs and EPSCs recorded under voltage-clamp conditions were similar to those for block of IPSPs and EPSPs recorded under current-clamp conditions. Moreover, voltage-clamp of neuronal membranes at their resting potential levels failed to prevent the MeHg-induced early increase in EPSC amplitude. In contrast, Hg2+ also blocked IPSPs more rapidly than it did EPSPs. However, it blocked IPSCs and EPSCs similarly when CA1 neuronal membranes were voltage-clamped at their resting potentials, suggesting that the early block by Hg2+ of IPSPs compared with that for EPSPs may be due simply to changes in resting membrane potential. Thus, MeHg blocked inhibitory synaptic transmission more preferentially, although it also blocked excitatory synaptic transmission, whereas Hg2+ blocked both inhibitory and excitatory transmission to the same extent and relatively slowly. This is consistent with our previous observations that MeHg caused early stimulatory effects on hippocampal synaptic transmission, although Hg2+ did not (Yuan and Atchison, 1994).

In dorsal root ganglion neurons, MeHg suppressed GABA-mediated chloride currents, although Hg2+ greatly enhanced these currents in a concentration-dependent manner (Ara-kawa et al., 1991; Huang and Narahashi, 1996). In our study, the IPSCs recorded at CA1 neurons appear to be primarily GABA_A-mediated chloride currents, because their reversal potentials are close to the equilibrium potential of Cl− and these currents can be blocked by bicuculline. At 20 and 100 μM, MeHg suppressed all inward and outward currents generated at different holding potentials and shifted the IV curve to more positive potentials, suggesting that MeHg may...
block the GABA<sub>A</sub>-mediated chloride channels. MeHg has also been shown to inhibit muscimol-stimulated agonist binding in cerebellar P<sub>2</sub> membrane fractions (Komulainen et al., 1995). In contrast, whereas Hg<sup>2+</sup> also suppressed to block all inward and outward Cl<sup>-</sup> currents, it took longer to do so than did MeHg. Unlike the effects of MeHg on GABA<sub>A</sub>-activated Cl<sup>-</sup> currents, Hg<sup>2+</sup> initially caused an increase in GABA<sub>A</sub>-mediated outward Cl<sup>-</sup> currents before suppressing them, indicating that Hg<sup>2+</sup> may, as it did to the GABA<sub>A</sub>-mediated chloride channels in dorsal root ganglion neurons (Arakawa et al., 1991; Huang and Narahashi, 1996), initially increase the open probability of GABA<sub>A</sub>-activated chloride channels. Moreover, similar to its effects on the tetrodotoxin-, bicuculline- and picrotoxin-insensitive slow inward currents induced in dorsal root ganglion neurons (Arakawa et al., 1991), Hg<sup>2+</sup> shifted the I/V curve and the reversal potential to more negative potentials, indicating that ions other than Cl<sup>-</sup> may be also involved. These differential effects of MeHg and Hg<sup>2+</sup> on GABA<sub>A</sub> receptors may explain why MeHg causes the early stimulatory effects on hippocampal synaptic transmission, although Hg<sup>2+</sup> does not.

If effects of MeHg on GABA<sub>A</sub> receptors are indeed responsible for the MeHg-induced early increase in population spike or EPSP amplitude, then pretreatment of slices with the GABA<sub>A</sub> antagonist bicuculline should eliminate the early increased phase in either population spikes or EPSPs. After pretreatment of slices with bicuculline, MeHg no longer caused an initial increase in population spike and EPSP amplitudes but still decreased them to block. The failure to induce the early increase in amplitude of population spikes was not due to a ceiling effect caused by bicuculline, although bicuculline significantly increased population spike amplitude to 180 to 200% of control. At the time bicuculline-stimulated amplitudes of population spikes reached maximal levels, increasing stimulation intensity still caused a further increase in population spike amplitude. Moreover, MeHg failed to cause the early stimulatory effects even under conditions in which the stimulation intensity was reduced to

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**Fig. 7.** Time course of effects of 100 μM MeHg on hippocampal pyramidal cell EPSPs evoked in the presence of bicuculline. EPSPs were recorded from a CA1 neuron of a hippocampal slice by subthreshold stimulation of Schaffer collaterals. After perfusion with 10 μM bicuculline for 30 min (from −30 to 0 min), EPSPs were increased greatly with multi-spikes on them (note that the vertical calibration bar for the trace at −10 min is different from that for traces at other time points). At 0 min, the stimulation intensity was reduced to a level that failed to evoke any spikes. The slice was then superfused with ACSF containing 100 μM MeHg and 10 μM bicuculline until complete block of EPSPs occurred. Each trace is a representative depiction of five recordings.

**Fig. 8.** Effects of 20 μM bicuculline and 100 μM MeHg on muscimol-evoked responses in a hippocampal pyramidal cell. Conventional intracellular microelectrode recordings were made in CA1 pyramidal cells in the presence of 20 μM AP-5 and 10 μM DNQX in ACSF. Bath application to slices of 100 μM (top) or 50 μM (bottom) muscimol for 15 sec caused a depolarization response. Top, Effects of application of 20 μM bicuculline for 10 min (B10 min) and 20 min (B20 min) on 100 μM muscimol-evoked responses. After the muscimol-evoked response was blocked completely (B20), washing the slice with bicuculline-free ACSF for 20 min (W20) partially reversed it. Bottom, Time course of effects of 100 μM MeHg on 50 μM muscimol-evoked response. Arrows indicate the starting point of application of muscimol. Each trace is a representative depiction of three to nine experiments. Calibration bars: vertical, 20 mV; horizontal, 100 second.
neurons, GABAB receptors are coupled to K+ channels via a G-protein to cause hyperpolarization of cells. This is expressed as the slow IPSP (Dutar and Nicoll, 1988b; Thompson et al., 1992; Otis et al., 1993; Pitler and Alger, 1994). Perhaps the delayed increase in population spike amplitude by MeHg-induced in the presence of bicuculline was due to an effect on GABA\textsubscript{A} receptors. Alternatively, effects of MeHg on intracellular Ca\textsuperscript{2+} homeostasis may also be involved in the early stimulatory effects of MeHg on hippocampal synaptic transmission, because MeHg increases intracellular Ca\textsuperscript{2+} concentrations in several types of neurons (Denny et al., 1993; Hare et al., 1993, 1995). In fact, in hippocampal slices after block of voltage-dependent Na\textsuperscript{+} channels using the local anesthetic QX-314, MeHg also caused an initial increase in Ca\textsuperscript{2+} spike amplitudes prior to decreasing them to block (Y. Yuan and W. D. Atchison, unpublished observation).

Earlier findings from ligand binding studies (Young and Snyder, 1973) and autoradiography (Zarbin et al., 1981; Frostholm and Rotter, 1985; Probst et al., 1986) using \[^{3}H\]strychnine indicated that glycine receptors are predominately confined to the spinal cord, brain stem and other areas of the lower neuraxis. However, recent studies using immunocytochemistry with monoclonal antibodies (Van den Pol and Gorcs, 1988; Becker et al., 1988), autoradiography with \[^{3}H\]glycine (Bristow et al., 1986), Northern blot hybridization (Grenningloh et al., 1990; Kuhse et al., 1990a; Malosio et al., 1991) and polymerase chain reaction (Kuhse et al., 1990a, 1990b, 1991) demonstrated a wide distribution of glycine receptors in the higher regions of the central nervous system including cerebral cortex and hippocampus. These glycine receptors, unlike those in the spinal cord and brain stem that primarily express the a1 subunit, a component of the “classical” strychnine-sensitive glycine receptor (Bristow et al., 1986; Becker et al., 1988; Belz, 1990), express a different ligand binding subunit (a2), which displays only low affinity for binding of strychnine (Bristow et al., 1986; Becker et al., 1988) or low sensitivity to strychnine upon heterologous expression in Xenopus oocytes (Kuhse et al., 1990a). However, to date we are unaware of any direct report of the existence and the physiological role of functional glycine receptors in hippocampal CA1 neurons, although the above evidence suggests their presence in the hippocampus. In our study, pretreatment of slices with strychnine caused a dramatic increase in population spike amplitude and induced multiple spike responses, although it was not as effective in this regard as was bicuculline. This suggests that there may be a small population of strychnine-sensitive subtype of glycine receptors located in the CA1 hippocampal region, or that strychnine cross-reacts with GABA\textsubscript{A} receptors, because they both belong to a superfamily of ligand-gated ion channels and share significant sequence similarity in primary structure and transmembrane topology (Grenningloh et al., 1987; Schofield et al., 1987; Langosch et al., 1988; Schmieden et al., 1993). The latter possibility seems less likely, inasmuch as strychnine did not prevent or suppress the MeHg-induced early stimulation, as did bicuculline pretreatment. Another possible explanation for the failure of strychnine pretreatment to block MeHg-induced early stimulation of synaptic transmission is that these heterologous glycine receptors in hippocampal neurons may be not blocked completely by strychnine due to their low sensitivity to strychnine as suggested by previous studies (Young and Snyder, 1973; Frostholm and Rotter, 1985; Probst et al., 1986; Bristow et al., 1986; Kuhse et al., 1990a). This may be one of the reasons
that strychnine was less potent in increasing population spike amplitude than was bicuculline. This possibility also seems less likely, because pretreatment of slices with bicuculline alone completely suppressed MeHg-induced early increase in field potentials. Thus, if there are glycine receptors located on postsynaptic membranes, they do not play a primary role in MeHg-induced early stimulation of hippocampal synaptic CA1 cell transmission.

In conclusion, the preferential block by MeHg of inhibitory synaptic transmission, mediated primarily by GABA_A receptors, appears to be primarily responsible for the MeHg-induced early stimulatory effects on hippocampal synaptic transmission. The importance of this disinhibition caused by MeHg to its overall neurotoxicity also remains unknown.

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