Comparative Effects of Methylmercury and Hg$^{2+}$ on Human Neuronal N- and R-Type High-Voltage Activated Calcium Channels Transiently Expressed in Human Embryonic Kidney 293 Cells

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ABSTRACT

Expression cDNA clones of α1E-1 or α1E-3 subunits coding for human neuronal N-(Ca$^{2+}$,v2.2) or R-subtype (Ca$^{2+}$,v2.3) Ca$^{2+}$ channels, respectively, was combined with α2.3δ and β3.4 Ca$^{2+}$ channel subunits, and transfected into human embryonic kidney cells for transient expression to determine whether specific types of neuronal voltage-sensitive Ca$^{2+}$ channels are affected differentially by methylmercury (MeHg) and Hg$^{2+}$. For both Ca$^{2+}$ channel subtypes, MeHg (0.125–5.0 μM) or Hg$^{2+}$ (0.1–5 μM) caused a time- and concentration-dependent reduction of current. MeHg caused an initial, rapid component and a subsequent more gradual component of inhibition. The rapid component of block was completed between 100 and 150 s after beginning treatment. At 0.125 to 1.25 μM, MeHg caused a more gradual decline in current. Apparent IC$_{50}$ values were 1.3 and 1.1 μM for MeHg, and 2.2 and 0.7 μM for Hg$^{2+}$ on N- and R-types, respectively. For N-type current, effects of Hg$^{2+}$ were initially greater on the peak current than on the sustained current remaining at the end of a test pulse; subsequently, Hg$^{2+}$ blocked both components of current. For R-type current, Hg$^{2+}$ affected peak and sustained current approximately equally. Kinetics of inactivation also seemed to be affected by Hg$^{2+}$ in cells expressing N-type but not R-type current. Washing with MeHg-free solution could not reverse effects of MeHg on either type of current. The effect of Hg$^{2+}$ on N- but not R-type current was partially reversed by Hg$^{2+}$-free wash solution. Therefore, different types of Ca$^{2+}$ channels have differential susceptibility to neurotoxic mercurials even when expressed in the same cell type.

Voltage-sensitive Ca$^{2+}$ channels play crucial roles in a number of cellular functions, including neurotransmitter release, gene expression, growth cone elongation, and dendritic action potential generation (Catterall, 1998, 2000). Various Ca$^{2+}$ channelopathies resulting in neuronal or neuromuscular disorders are caused by mutations in genes coding for Ca$^{2+}$ channel subunits (for review, see Meir and Dolphin, 2002). At least six distinct subtypes of Ca$^{2+}$ channels (L, N, T, P, Q, and R) have already been identified based on their differential biophysical, molecular biological, and pharmacological properties (Tsien et al., 1995). Neuronal Ca$^{2+}$ channels contain four subunits: α$_1$, β, α$_2$, and δ. The α$_1$ subunit is the pore-forming, voltage-sensing, and ligand-binding component. cDNAs for at least seven distinct α subunits for high-voltage activated Ca$^{2+}$ channels: α1A-1F, α1S, have been cloned. Four different β subunits and two different α$_2$ subunits regulate assembly and modulate the kinetic parameters of the channel. The presence of several isofoms and splice variants further complicates the functional expression characteristics and classification of high-voltage activated Ca$^{2+}$ channels (Brust et al., 1993; DeWaard and Campbell, 1995; McEnery et al., 1998; Pan and Lipscombe, 2000). Cells typically coexpress several types of Ca$^{2+}$ channels, often with similar subcellular localization, providing a highly regulated degree of control over Ca$^{2+}$-dependent cell functions, but confounding analyses of the properties of distinct Ca$^{2+}$ channel subtypes in isolation. Because of their portal location within the plasma membrane, Ca$^{2+}$ channels are potentially susceptible to the actions of a number of polyvalent heavy metal-type toxicants and serve as entry paths into the cell for heavy metals (Kiss and Osipenko, 1994; Atchison, 2003).
Because of the crucial roles that Ca\(^{2+}\) channels play in key cellular functions, toxicant effects on Ca\(^{2+}\) channels could have significant deleterious consequences for neuronal function.

Methylmercury (MeHg) and inorganic mercury (Hg\(^{2+}\)) are environmental neurotoxins that differ chemically in ionic charge, ionic radii, and lipophilicity. Together, these factors can impact the manner in which these mercurials affect a given cellular function. Neurotoxic mercurials act on a number of cellular targets. In several neuronal systems, cellular effects of MeHg and Hg\(^{2+}\) are similar, yet distinct (Atchison et al., 1986; Hare and Atchison, 1992; Hewett and Atchison, 1992; Yuan and Atchison, 1994). The exact mechanisms by which these mercurials exert neurotoxicity are not known with certainty.

Disruption of function of voltage-sensitive Ca\(^{2+}\) channels is a prominent effect of acute exposure to low concentrations of both MeHg (Shafer and Atchison, 1991; Leonhardt et al., 1996; Sirois and Atchison, 1996, 2000; Shafer, 1998) and Hg\(^{2+}\) (Büsselberg et al., 1991; Weisenberg et al., 1995). MeHg blocks Ba\(^{2+}\) currents (I\(_{Ba}\)) carried through multiple subtypes of Ca\(^{2+}\) channels in primary cultures of cerebellar granule cells and in rat pheochromocytoma (PC12) cells (Shafer and Atchison, 1991; Sirois and Atchison, 2000). Hg\(^{2+}\) also alters function of several types of Ca\(^{2+}\) channels at low micromolar concentrations (Büsselberg et al., 1994; Leonhardt et al., 1996; Szucs et al., 1997). However, the actions of mercurials on Ca\(^{2+}\) channels may be more complex than mere block of function. In PC12 cells, very low concentrations of Hg\(^{2+}\) increase amplitude of current carried through voltage-sensitive Ca\(^{2+}\) channels (Rossi et al., 1993), whereas in cerebellar granule cells and NG108-15 cells, MeHg causes an increase in fura-2 fluorescence, which is dependent, at least in part, on extracellular Ca\(^{2+}\), and which is delayed by nifedipine, \(\omega\)-conotoxin GVIA, and Ni\(^{2+}\) (Hare and Atchison, 1995; Marty and Atchison, 1997). Moreover, treatment of rodents with Ca\(^{2+}\) channel blockers prevents the toxic effects of MeHg (Sakamoto et al., 1996), and Ca\(^{2+}\) channel blockers delay the onset of cerebellar granule cell death with MeHg (Marty and Atchison, 1997; Gasso et al., 2001). Finally, in cells lacking Ca\(^{2+}\) channels, the onset of intracellular action of MeHg is delayed, suggesting that Ca\(^{2+}\) channels provide a path of entry for MeHg into the cell (Edwards et al., 2002). Therefore, mercurials seem to interact with voltage-sensitive Ca\(^{2+}\) channels in a complex manner.

Because of the numerous and important roles that voltage-sensitive Ca\(^{2+}\) channels play in neuronal function, disruption of function of voltage-sensitive Ca\(^{2+}\) channels may be a significant contributory factor in mercurial-induced neurotoxicity. There are few published reports comparing the effects of different mercurials on function of voltage-sensitive Ca\(^{2+}\) channels (Hewett and Atchison, 1992; Szucs et al., 1997; Schirrmacher et al., 1998), and no comparative study on the effects of these two forms of mercury on defined types of voltage-sensitive Ca\(^{2+}\) channels exists.

The goal of the present study was to determine whether specific types of voltage-sensitive Ca\(^{2+}\) channels were affected differentially by MeHg or Hg\(^{2+}\). We compared the effect of MeHg and Hg\(^{2+}\) on N\(_{a}\)(Ca\(_{a,2}\)) and B-(Ca\(_{a,2}\)) types of voltage-sensitive Ca\(^{2+}\) channels expressed using cDNA copies of their genes transferred into human embryonic kidney cells (HEK293). These cells are nonexcitable and commonly used for heterologous expression of membrane proteins, including voltage-dependent Ca\(^{2+}\) channels (Williams et al., 1994; Perez-Garcia et al., 1995; Quefurth et al., 1998). Expression cDNA clones of \(\alpha_1\)I-\(\alpha_3\) or \(\alpha_1\)E-\(\beta_3\) subunit were combined with \(\beta_2\)\(\beta_3\) or \(\beta_3\)\(\beta_3\) Ca\(^{2+}\) channel subunits of human neuronal origin for transient expression of N- and R-subtypes, respectively, of high-voltage activated Ca\(^{2+}\) channels. Jellyfish green fluorescent protein (GFP) was used as a cotransfection reporter.

Materials and Methods

Materials. HEK293 cells (ATCC CRL-1573) were purchased from American Type Culture Collection (Manassas, VA). All reagents were pure or ultrapure laboratory grade unless specifically noted otherwise. cAMP, EGTA, HEPES, ATP, \(\omega\)-conotoxin GVIA, and tetrodotoxin were all obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions (5 mM) of methylmercuric chloride (ICN Pharmaceuticals, Costa Mesa, CA) and (10 mM) HgCl\(_2\) were prepared weekly in double-distilled water, from which test solutions were prepared daily in extracellular solution (see below). Plasmids containing expression cDNA clones of human neuronal Ca\(^{2+}\) channel subunits were generously provided by Dr. Kenneth A. Stauderman of SIBIA Neurosciences (San Diego, CA), now Merck Research Laboratories. \(\alpha_1\)E-\(\beta_3\) (Williams et al., 1994) and \(\beta_2\)\(\beta_3\) subunit clones (Mark Williams, Merck Research Laboratories, personal communication) were isolated from hippocampus; \(\alpha_1\)I-\(\beta_3\) was isolated from the IMR32 cell line (Williams et al., 1992b), and \(\alpha_2\) was isolated from brainstem and basal ganglia (Williams et al., 1992a).

Cell Culture and Transfection. HEK293 cells were grown at 37°C in Eagle's minimal essential medium fortified with 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM l-glutamine, 1.5 g/l sodium bicarbonate, 10% (v/v) fetal bovine serum, and penicillin/streptomycin/amphotericin B (at a final concentration of 100 U/ml penicillin G, 100 \(\mu\)g/ml streptomycin sulfate, and 0.25 \(\mu\)g/ml amphotericin B; Invitrogen, Carlsbad, CA) in a 5% CO\(_2\) environment. One day before gene transfer, cells were plated at a density of 5 \(\times\) 10\(^4\) on 35-mm culture dishes. Cells were transfected with a mixture of plasmids containing human neuronal \(\alpha_1\)E-\(\beta_3\) or \(\alpha_1\)I-\(\beta_3\) plus \(\alpha_2\)\(\beta_3\) or \(\beta_3\)\(\beta_3\) Ca\(^{2+}\) channel subunits and a jellyfish GFP cDNA sequence using FuGENE 6 (Roche Diagnostics, Indianapolis, IN) following the manufacturer's instruction. Reactions contained a total of 3 \(\mu\)l of FuGENE 6 and 1 \(\mu\)g of plasmid DNA containing the three subunits in a 1:1:1 ratio, with GFP plasmid at 20% of the total DNA. Two days were allowed for transient expression of proteins at which time the cells were examined for GFP expression. Cells from dishes with a reasonable number of green fluorescent cells (usually 20%), were replated at a low density of sufficiently isolated individual cells to facilitate recording.

Ca\(^{2+}\) Channel Current Recording. Before recording, culture medium was removed, cells were rinsed twice with extracellular solution, and then replenished with 1 ml of extracellular recording solution. The extracellular solution contained 117 mM tetraethylammonium chloride, 20 mM BaCl\(_2\), 1 mM MgCl\(_2\), 25 mM d-glucose, 10 mM HEPES, and 0.001 mM tetrodotoxin; pH was adjusted to 7.2 at room temperature (23–25°C) with tetraethylammonium hydroxide. The osmolarity of the solution was 310 mosM. Patch-clamp pipettes with resistances between 6 and 8 MΩ were prepared from 1.5-mm i.d. glass capillaries (WPI, Sarasota, FL) using a two-stage microelectrode puller (PP-830; Narishige, Tokyo, Japan) and fire-polished using a Narishige (PP-830) microforge. Intracellular (pipette) solution contained 140 mM CsCl, 10 mM EGTA, 10 mM HEPES, and 0.901 mM tetrodotoxin; pH was adjusted to 7.2 at room temperature (23–25°C) with tetraethylammonium hydroxide.

The tight seal whole cell configuration of the patch-clamp technique (Hamill et al., 1981) was used on fluorescent green cells to record I\(_{Ba}\) through transiently expressed Ca\(^{2+}\) channels. Whole cell currents were recorded using an Axopatch-1D amplifier (Axon In-
Results

Characteristics of Recombinant Transiently Expressed \( \alpha_{1B} \) and \( \alpha_{1E} \) Subunit-Mediated \( \text{Ca}^{2+} \) Channel Currents in HEK293 Cells. Basic biophysical and pharmacological qualities of \( \text{Ca}^{2+} \) channels containing \( \alpha_{1B} \) (Ca\(_{\text{V}2.2}\)) or \( \alpha_{1E} \) (Ca\(_{\text{V}2.3}\)) subunit in combination with \( \alpha_{2-b} \delta \) and \( \beta_{3-a} \) subunits and expressed transiently in HEK293 cells were similar to those of N- or R-type \( \text{Ca}^{2+} \) channels, respectively, as expressed in their native environment. A constant set of \( \alpha_{2-b} \delta \) and \( \beta_{3-a} \) subunits was used in all experiments so that we could focus on the comparative actions of the two mercurials on the principal phenotype-defining subunit of voltage-sensitive \( \text{Ca}^{2+} \) channels, the \( \alpha_1 \) subunit. Representative tracings of \( I_{\text{Ba}} \) elicited from these cells along with their current-voltage relationships and voltage dependence of steady-state inactivation and activation are shown in Fig. 1, A to C (for \( \alpha_{1B} \)) and Fig. 2, A to C (for \( \alpha_{1E} \)), respectively. For \( \alpha_{1B} \)-containing channels, current seemed to activate at approximately \(-20 \) mV, reached peak amplitude at about \(+20 \) mV and reversed at \(+60 \) mV. For channels containing \( \alpha_{1E} \) subunit, current activated at \(-40 \) mV, reached a peak amplitude at \(0 \) mV and reversed at approximately \(+60 \) mV. The voltage for half-maximal activation (\( V_{1/2} \)) was \(0 \) mV in \( \alpha_{1B} \)-containing channels (Fig. 1C) and \(-14.8 \) mV in \( \alpha_{1E} \)-containing channels (Fig. 2C), respectively. The isochronal inactivation (\( h \)) was determined using an 8-s inactivating prepulse. The inactivation curves are shown (Figs. 1C and 2C) with slope parameter (\( k \)) of 12.0 mV and \( V_{1/2} \) of \(-64.6 \) mV for \( \alpha_{1B} \)-containing channels.

![Fig. 1](image-url) Biophysical characteristics of whole cell \( \text{Ba}^{2+} \) current mediated by recombinant N-type \( \text{Ca}^{2+} \) channels expressed in HEK293 cells. A, representative family of current traces (filtered at 2 kHz but not leak subtracted) elicited by voltage steps from a holding potential of \(-90 \) mV to test potentials ranging from \(-20 \) to \(+60 \) mV. \( \text{Ba}^{2+} \) \((20 \) mM\) was used as the charge carrier. B, current-voltage relationship for the \( \text{Ba}^{2+} \) current was derived from the type of experiment illustrated in A. The peak current elicited during a voltage step is plotted as a function of the test potential. C, voltage dependence of activation (○) and of steady-state inactivation (□) is depicted. To examine voltage dependence of activation, peak current elicited during a depolarizing voltage step from the holding potential to each test potential was normalized to the maximum current elicited and normalized current was plotted as a function of test potential. Data points were fitted by the curve using a Boltzmann function: 
\[
I/\text{max} = [1 + \exp((V - V_{1/2})/k)]^{-1},
\]
with \( V_{1/2} = -64.6 \) mV and \( k = 12.0 \) mV. To examine the voltage dependence of steady-state inactivation, a conditioning voltage step (8 s, to potentials between \(-100 \) and \(0 \) mV, in 10-mV increments) was applied to allow inactivation of the channel to achieve steady state, and the membrane was then stepped to \(+20 \) mV. Current elicited at \(+20 \) mV after each conditioning step was measured and normalized to amplitude elicited (at \(+20 \) mV) after a conditioning step to \(-100 \) mV. Normalized current was plotted as a function of conditioning (step) potential. Data points were fitted by the curve using a Boltzmann function: 
\[
I/\text{max} = [1 + \exp(-(V - V_{1/2})/k)]^{-1},
\]
with \( V_{1/2} = 0.0 \) mV and \( k = 3.6 \) mV. \( \text{Ba}^{2+} \) current amplitude was measured after subtraction of leak current from the whole current elicited by the activating depolarization. In B and C, values shown are the mean ± S.E.M. of 10 cells.
and $k$ of 9.2 mV and $V_{1/2}$ of −69.6 mV for $\alpha_{1B}$-containing channels, respectively.

Figure 3 demonstrates that $I_{Ba}$ expressed in cells transfected with $\alpha_{1B}$ subunit was sensitive to inhibition by $\omega$-conotoxin GVIA. At 1 or 10 μM, $\omega$-conotoxin GVIA caused a rapid reduction of current amplitude; the rate with which block occurred was concentration-dependent. However, ultimately, both concentrations blocked virtually all current. The effect of $\omega$-conotoxin block was not reversed by washing the cell with toxin-free solution. Thus, the $I_{Ba}$ recorded from HEK293 cells expressing human neuronal $\alpha_{1B}$ subunits combined with a fixed complement of $\alpha_{2,3}$$\delta_{3}$ subunits has biophysical and pharmacological properties consistent with those mediated by N-type Ca$^{2+}$ channels. We tested $\alpha_{1B}$ subunit-expressing HEK293 cells with known specific antagonists (nimodipine and $\omega$-agatoxin IVA) for the L- or P/Q-type channels and found the $I_{Ba}$ to be resistant to block by both of these agents (data not shown), consistent with the characteristics of R-type current. Therefore, we used varying concentrations of Cd$^{2+}$ and Ni$^{2+}$ (both of which are known to block current carried through all known subtypes of voltage-sensitive Ca$^{2+}$ channels) on the $\alpha_{1B}$ subunit-containing cells (Fig. 4, A and B). The block of $I_{Ba}$ from these cells by both divalent cations was concentration-dependent. A stepwise reduction of current was seen after addition of successively higher concentrations of Cd$^{2+}$ or Ni$^{2+}$; this effect seemed to plateau until the next higher concentration of metal was added. The reduction of amplitude of $I_{Ba}$ was complete at 100 μM Cd$^{2+}$ or 1 mM Ni$^{2+}$. These two observations together indicate that the pharmacological characteristics of $\alpha_{1B}$ subunit-mediated $I_{Ba}$ from these cells is consistent with that of the R-type. An interesting observation at the lowest concentrations of Cd$^{2+}$ (0.1 μM; Fig. 4A) and Ni$^{2+}$ (1 μM; Fig. 4B) was an apparent slight increase rather than reduction in amplitude of $I_{Ba}$. This effect was more prominent with Cd$^{2+}$.
Traces, and in composite data from a number of preparations.

A subunit-containing channels. As shown in Fig. 5A for cells expressing human neuronal α_{1E} subunit-containing Ca^{2+} channels. Notice the stepwise reduction of current amplitude with each higher concentration of Cd^{2+} or Ni^{2+} addition that seems to plateau until the next higher concentration is added. The block of Ba^{2+} current becomes complete at 100 μM Cd^{2+} and 1 mM Ni^{2+}. Cells were depolarized from −90 to 0 mV at a stimulation frequency of 0.1 Hz. Current responses were filtered at 2 kHz and leak current was subtracted. Values shown are the mean ± S.E.M. of six to seven cells.

Perhaps this effect is due to these cations actually entering the Ca^{2+} channels based on their relatively similar ionic radii and electrical charges. These same cations become inhibitory at higher concentrations.

**Time- and Concentration Dependence of MeHg Effect on I_{Ba}** As shown in Figs. 5A and 6A for representative traces, and in composite data from a number of preparations in Figs. 5B and 6B, MeHg caused a rapid, concentration- and time-dependent reduction in current from both α_{1B} and α_{1E} subunit-containing channels. As shown in Fig. 5A for cells expressing α_{1B}-type current, the effect of 5 μM MeHg was initially greater on the peak current than on the sustained current remaining at the end of a test pulse. However, with additional time, MeHg blocked both components of current apparently equally. For cells expressing α_{1E} subunit, the degree of block of current by 5 μM MeHg seemed to be approximately the same for both peak and sustained current (Fig. 6A). The effect of different concentrations of MeHg on whole cell I_{Ba}, with either subunit is shown as a time course in Figs. 5B and 6B and was determined after at least 5-min stabilization of the control current. Addition of MeHg concentrations between 0.125 (or 0.25 for α_{1E} subunit) and 5.0 μM to the extracellular solution resulted in a concentration-dependent block in the rate and magnitude of I_{Ba} after leak subtraction. For both kinds of channels, the block of peak current by MeHg was again concentration- and time-dependent. MeHg concentrations in excess of 1 μM caused complete block of current within 2 to 6 min. Submicromolar concentrations also caused measurable inhibition (Figs. 5B and 6B). The rate at which inhibition occurred at micromolar concentrations seemed to consist of two distinct components: one which was initially more rapid, and the other, which was more gradual (Figs. 5B and 6B). Depending upon the concentration of MeHg used, the rapid component was completed between 100 and 150 s after beginning treatment. Lower concentrations of MeHg (0.125–1.25 μM) caused a more gradual decline in current amplitude. Inhibition of current at 1.25 μM MeHg seemed ultimately to reach the same plateau as at higher concentrations. At 0.125 μM in α_{1B} and 0.25 μM in α_{1E}-containing channels, inhibition never reached the same level as at higher concentrations over the 10-min recording period. Because lower concentrations of MeHg caused a slow, progressive block of current that didn’t reach a plateau, we could only estimate an apparent IC_{50} value for MeHg. This was done by comparing the percentage of block at each concentration at a set time point. At 200 s, MeHg blocked ~50% of both types of current at 1.3 (N-type) and 1.1 μM (R-type).

**Concentration Dependence of Hg^{2+} Effect on I_{Ba}** Figures 7 and 8 show the effects of Hg^{2+} on I_{Ba} from HEK293 cells expressing α_{1B} and α_{1E} subunit-containing Ca^{2+} channels, respectively. Figures 7A and 8A depict representative current traces showing effects of different concentrations of Hg^{2+} on current after 2-min exposure. Hg^{2+} caused a rapid, concentration-dependent reduction in current from both α_{1B} and α_{1E} subunit-expressing channels. As shown in Fig. 7A for cells expressing α_{1B}-type current, the effect of 0.5, 1, and 5.0 μM Hg^{2+} was initially greater on the peak current than on the sustained current remaining at the end of a test pulse.
However, with additional time, Hg²⁺ blocked both components of current. For cells expressing α₁E subunit, the degree of block by 0.1, 0.5, and 1 μM Hg²⁺ seemed to be approximately the same for both peak and sustained current (Fig. 8A). Furthermore, the inactivation kinetics seems to be affected by Hg²⁺ in cells expressing α₁E-subunit current. Figures 7B and 8B depict the degree of reduction of normalized Iₚ at a stimulation frequency of 0.1 Hz as a function of concentration of Hg²⁺. The IC₅₀ values for Hg²⁺-induced block of α₁B and α₁E-type current were 2.2 and 0.7 μM, respectively.

**Effect of Wash with MeHg- or Hg²⁺-Free Solution on Iₚ.** As shown in Fig. 9, the block by MeHg of either type of current could not be reversed by washing with MeHg-free solution. However, the Hg²⁺ induced reduction of Iₚ through α₁B-mediated N-type channels was partially reversible (Fig. 10A), but the Iₚ reduction from α₁E-mediated R-type channels was not reversed by washing with Hg²⁺-free solution (Fig. 10B).

**Discussion**

Previous studies have shown that MeHg (Shafer and Atchison, 1991; Marty and Atchison, 1997; Shafer, 1998; Siros and Atchison, 2000) and Hg²⁺ (Büsselberg et al., 1994; Leonhardt et al., 1996; Szucs et al., 1997) affect the function of native Ca²⁺ channels in multiple types of cells. However, in most of these studies, the cells examined express more than one subtype of Ca²⁺ channels, and attempts to compare the actions of these metals on known subtypes of Ca²⁺ channel were incomplete and/or indirect. Until recently (Peng et al., 2002), there have been no studies of effects of mercurials on distinct subtypes of Ca²⁺ channel in isolation. The present study using transient expression of human neuronal Ca²⁺ channels is the first to characterize and compare the effects of the organomercurial MeHg and inorganic Hg²⁺ on current mediated by single, identified phenotypes (N- and R-type) of Ca²⁺ channel in isolation. Because the two types of expressed Ca²⁺ channel contained the same α₂δ and β subunits, differential effects of the mercurials on expressed N- and R-type
channel current must be due largely to the mercurial interactions with, or action on, the \( \alpha_1 \) pore-forming subunit of the channel. Our results support and extend several aspects of previous studies of mercurials on native N-type and R-type \( \text{Ca}^{2+} \) channels. Our results demonstrate that, first, MeHg is an irreversible and essentially equipotent inhibitor of current recorded from HEK293 cells expressing human neuronal \( \alpha_{1 \text{B}} \) (N-type) or \( \alpha_{1 \text{E}} \) (R-type) subunit containing \( \text{Ca}^{2+} \) channels. Second, although \( \text{Hg}^{2+} \) inhibits current mediated by the same channels, it has a more potent effect on recombinant R-type channels than N-type channels. Third, the effect of \( \text{Hg}^{2+} \) is partially reversible for recombinant N-type channels but is evidently irreversible for R-type channels.

Our recordings show that the voltage- and time-dependent characteristics of the current mediated by channels containing the \( \alpha_{1 \text{B}} \) subunit and those containing the \( \alpha_{1 \text{E}} \) subunit expressed in transfected cells resemble those of native N- and R-type \( \text{Ca}^{2+} \) channels, respectively, in neurons (Randall and Tsien, 1995). The different biophysical characteristics of native N-type channel current and current mediated by recombinant channels containing the \( \alpha_{1 \text{B}} \) subunit and of native R-type channel current and current mediated by recombinant channels containing the \( \alpha_{1 \text{E}} \) subunit may simply reflect differences of the \( \alpha, \delta \) and \( \beta \) subunits expressed in recombinant and native channels. Alternatively, these differences may be due to differences of the human \( \alpha \) subunits expressed in cells studied here and the \( \alpha_1 \) subunits of the native channels of nonhuman derived neurons such as those studied by Randall and Tsien (1995). Furthermore, we have demonstrated that recombinant channels containing the \( \alpha_{1 \text{B}} \) subunit are sensitive to \( \omega \)-conotoxin GVIA as are native N-type \( \text{Ca}^{2+} \) channels, whereas those channels expressing the \( \alpha_{1 \text{E}} \) subunit were highly sensitive to block by \( \text{Cd}^{2+} \) and to a lesser extent \( \text{Ni}^{2+} \) as is the case for native R-type channels.

The data presented here show that recombinant expressed N-type channels were more sensitive to the effects of MeHg than were presumptive N-type native channels in PC12 cells (Shaffer and Atchison, 1991; Shaffer, 1998) and dorsal root ganglion cells (Leonhardt et al., 1996) but were equally sensitive compared with presumptive N-type native channels in PC12 cells. Another difference between the effects of MeHg on recombinant and native channels is that \( \text{Hg}^{2+} \) seems to block current through native channels at lower concentrations (Manalis and Cooper, 1975; Rossi et al., 1993); this phenomenon was not observed in our study of recombinant channels.

In summary, both MeHg and \( \text{Hg}^{2+} \) perturb the function of heterologously expressed, recombinant human neuronal N-type and R-type \( \text{Ca}^{2+} \) channels at low micromolar concentrations, well within the range of concentrations known to cause disruption of function of corresponding native channels as well as toxicity from these agents in vivo. MeHg was an equipotent inhibitor of human neuronal N-type and R-type \( \text{Ca}^{2+} \) channels expressed in HEK293 cells. However, there seem to be subtle differences on the effects of \( \text{Hg}^{2+} \), which vary somewhat depending on the type of \( \alpha_1 \) subunit.

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