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Mercuric Ions Are Potent Noncompetitive Antagonists of Human Brain Kainate Receptors Expressed in *Xenopus* Oocytes

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SUMMARY

Kainate receptors are one of the major subtypes of excitatory amino acid receptors in the vertebrate central nervous system. Using *Xenopus* oocytes injected with RNA from human temporal cortex, it is possible to detect electrophysiologically the expression of this receptor subtype in these cells. Ions of the group IIb elements, particularly mercuric ions, are highly potent, noncompetitive inhibitors of these human brain kainate receptors. Mercury-containing sulfhydryl reagents are also very effective, irreversible blockers of the kainate-gated currents of these oocytes. The recovery of kainate-activated currents after washout of Hg^{2+} is slow and incomplete relative to that seen after treatment either with Cd^{2+} or Zn^{2+}. Cysteine or dithiothreitol can accelerate this recovery of kainate-inducible currents after Hg^{2+} inhibition. Besides the toxicological implications of these results, mercury compounds may be useful for future studies of the structure and physiology of the kainate receptor-channel complex.

The diacidic amino acids, glutamate and aspartate, are potent excitants of most vertebrate central neurons (1–3). At least three receptor subtypes are postulated to mediate neuronal responses to these ligands (4–6). These receptor subtypes are distinguished both conceptually and experimentally by the amino acid analogs, kainate, NMDA, and quisqualate, that selectively activate them. In addition, NMDA receptors appear to be modulated by a variety of endogenous and exogenous ligands such as glycine (7), dissociative anesthetics (8, 9) like ketamine, and the divalent cations Mg^{2+} and Zn^{2+} (10–13). In view of the potent effects of Mg^{2+} and Zn^{2+} on NMDA receptors, we were prompted to assess the effects of related divalent cations on the kainate-evoked responses of *Xenopus* oocytes injected with human brain RNA. These studies culminated in the finding that Hg ions are extremely potent, noncompetitive inhibitors of the kainate-triggered currents of these RNA-injected oocytes.

This demonstration of heavy metal ion inhibition of the kainate-evoked response of oocytes confirms and extends related observations by several other groups. Kiskin and coworkers (14) documented the noncompetitive blockade of kainate-gated currents of rat hippocampal neurons by Hg^{2+} and thiol-modifying reagents. An investigation using oocytes injected with rat brain mRNA showed that Zn^{2+} and Cd^{2+} were noncompetitive antagonists of kainate-triggered responses (15). Thirdly, Terramani and colleagues (16) reported the effects of thiol reagents on the binding of ligands to different glutamate receptor subtypes of rat synaptic membranes. Taken together, these results indicate that thiol groups of specific glutamate receptor subtypes play important roles either in modulating ligand binding or in regulating the opening of the agonist-controlled ion channels.

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Materials and Methods

Preparation of RNA and injection and culturing of oocytes. Total RNA was isolated by a chloroform-phenol procedure from a 2.8-g segment of human temporal cortex that had been surgically removed in the treatment of epilepsy (see Ref. 17). *Xenopus* oocytes (1.2–1.5 mm) were injected with 40–80 ng of this RNA, collagenase treated, and manually defolliculated in preparation for voltage-clamp recording, as described before (17). Recordings were normally obtained 4–7 days after RNA injection of the oocytes.

Transmembrane current measurements. Transmembrane currents of oocytes were measured using two-electrode voltage-clamp at 20–22°. The membrane of the oocyte was normally held at −60 mV and measurements were made of the current elicited by bath application of kainic acid in normal frog Ringer solution (pH 7.2–7.4). Only the RNA-injected oocytes exhibited detectable responses to kainate (1 mM) (see also Refs. 15 and 18).

Solutions and reagents. Normal frog Ringer solution contained (in mM): NaCl (110), KCl (2), $CaCl_2$ (1.8), and HEPES (10), with the pH adjusted to 7.2–7.4 with 1 M NaOH. The following reagents were purchased from Sigma: kainic acid, *N*-ethylmaleimide, dithiobisnitrobenzoic acid, PCMB, iodoacetamide, NMDA, quisqualic acid, DL-2-

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3, dione; AMPA, (*R,S*)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; PCMB, *p*-chloromercuribenzenesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

amino-5-phosphonopentanoic acid, and mersalyl acid. AMPA and CNQX were from Tocris Neuramin.

Results

Response of oocytes to kainate. In Fig. 1A are presented typical responses to kainic acid of an oocyte injected with RNA from human temporal cortex. The kainate-triggered inward current develops promptly and is well sustained throughout the period of ligand application. It then returns rapidly to baseline when the agonist is removed (Fig. 1A). This kainate-evoked current is completely blocked by the recently described antagonist (19) CNQX at 10 μM (Fig. 1B). At 1 μM, CNQX still inhibits more than 90% of the current evoked by 0.1 mM kainate, indicating that this ligand is a potent blocker of human brain kainate receptors. Assessment of the pA_2 for CNQX inhibition of kainate-gated current (Fig. 1C) gives a value of 6.7. The current-voltage relation for the kainate-induced current (Fig. 1C) shows a reversal of polarity at −12 mV. For 16 oocytes, the mean (± SD) reversal potential in normal frog Ringer was −10 ± 4 mV. This value is similar to those obtained previously for kainate-evoked currents seen either in oocytes (15, 18, 20, 22) or in vertebrate neurons (23–26).

We performed several experiments to ensure that the kainate-evoked responses we recorded using the RNA-injected oocytes were mediated solely via the kainate subtype of glutamate receptor and not by NMDA or quisqualate receptors. First, whereas the threshold for kainate-evoked responses was about 20 μM (see below), neither quisqualate, AMPA, nor NMDA (up to 1 mM) elicited any appreciable membrane response in these oocytes (in no instance did any one of these ligands elicit a current in excess of 1 nA in 10 RNA-injected oocytes from three different frogs). However, it is important to note that other RNA preparations have been shown to induce oocytes to develop responsiveness to these ligands (18, 21, 22) and we have detected typical responses (18) to these agonists in oocytes injected with other preparations of human brain mRNA. However, to minimize any complications that could arise from kainate action on other types of glutamate receptors, these experiments were confined to oocytes injected with the one preparation of RNA that induced detectable expression only of kainate receptors.

We also tested whether ligands that are known to modulate NMDA receptors (see Introduction) had any influence on the kainate-triggered currents in these cells. The racemic mixture of 2-amino-5-phosphonopentanoic acid (0.1 mM), the D-form of which is a potent NMDA receptor antagonist (4, 5), did not affect currents elicited by 1 mM kainic acid. Similarly, glycine (1 mM) and Mg^{2+} (1 mM) produced no modification of the kainate-evoked response of these oocytes. However, as observed before (18, 23, 24), other glutamate receptor agonists, like quisqualate, attenuate the current evoked by 1 mM kainate (in the presence of 10 μM quisqualate, the kainate current was diminished 60 ± 3%; $n = 3$). Thus, even though quisqualate does not, by itself, gate any current in these oocytes, it appears to interfere with agonist binding at the kainate recognition site.

Metal ion inhibition of kainate receptors. Hg^{2+} antagonizes kainate responses of rat hippocampal neurons (14) and, because Zn^{2+} and Cd^{2+} were recently shown to be noncompetitive blockers of kainate-evoked currents of oocytes injected with rat brain mRNA (19), we tested whether any of the group IIb metal cations affected the kainate response of oocytes

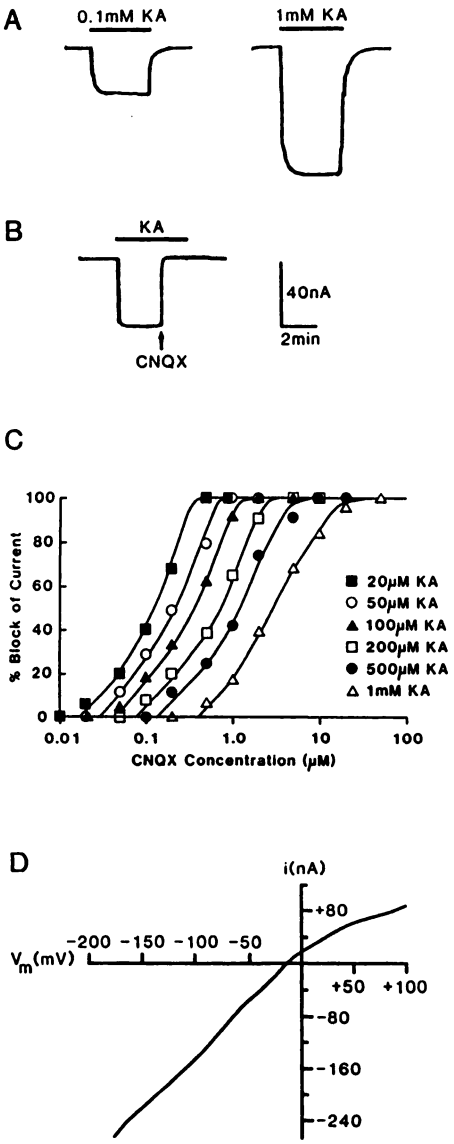

Fig. 1. A, Response of an oocyte to 0.1 or 1 mM kainate (*KA*) in normal frog Ringer solution (the presence of kainate in this and other figures is indicated by the *solid line*). The oocyte was injected with human brain RNA and currents were measured under voltage clamp. Same scale as in B. B, CNQX (0.01 mM) applied during a response evoked by 0.1 mM kainate completely blocks the kainate-triggered current. C, pA_2 analysis for CNQX antagonism of kainate-gated current. The percentage of inhibition of kainate-evoked currents is plotted as a function of CNQX concentration for different levels of kainate. A Schild plot was used to determine the pA_2 (36). D, Current-voltage relation for the kainate-evoked current of an oocyte injected with human brain RNA. The current-voltage relation was determined by passing 2-sec voltage ramps from −200 to +100 mV (from V_H = 50 mV) in frog Ringer solution with and without kainate (1 mM). The reversal potential was determined as the point at which the two current ramps intersected. These ramps were subtracted to produce the *I-V* relation for the kainate-evoked current.

injected with human brain RNA. As illustrated in Fig. 2A, Zn^{2+}, Cd^{2+}, and Hg^{2+} are all capable individually of fully arresting the current evoked by kainate at 1 mM. The blockade of the kainate-triggered current is obtained using 1 μM Hg^{2+}, whereas millimolar levels of Cd^{2+} or Zn^{2+} are used to produce the same degree of block. This issue of potency is addressed in more detail below.

Abolition of the kainate-gated current can also be achieved

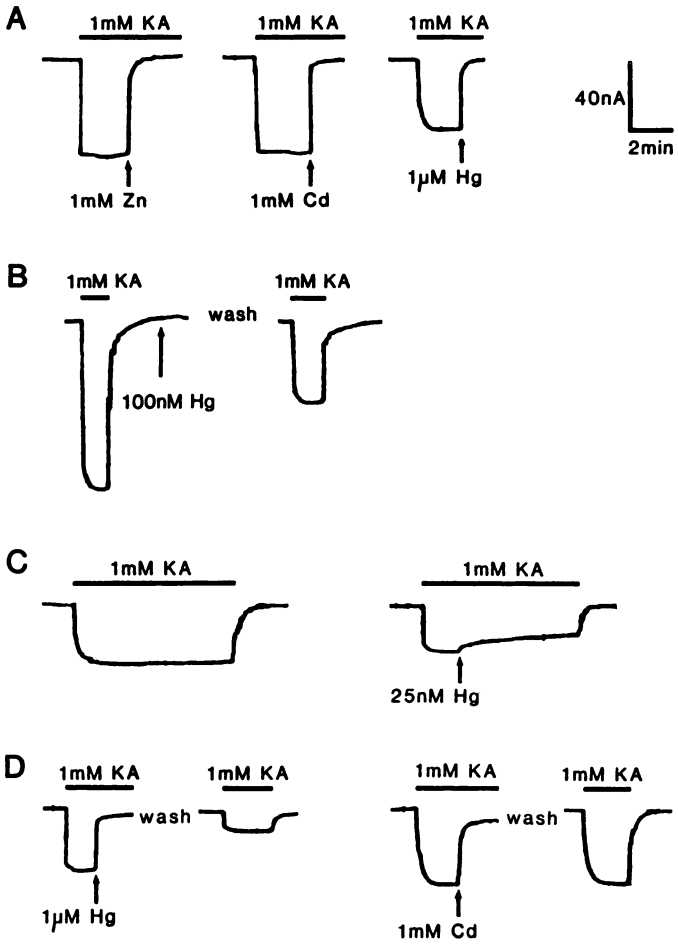

Fig. 2. A, Examples of the blockade of kainate (*KA*) responses by Zn^{2+}, Cd^{2+}, and Hg^{2+}. Stable responses to kainate (1 mM) are inhibited by inclusion of the indicated concentration of metal ion in the Ringer solution in the continued presence of agonist. B, Prior exposure of an oocyte to Hg^{2+} inhibits the response to a subsequent application of kainate (1 mM). The bath solution was exchanged 10 times before the second challenge with kainate. C, Fast and slow components of metal ion action. Kainate responses exhibit little or no desensitization to applied agonist on a time scale of minutes (*left*). When a low concentration of Hg^{2+} is added to the bath solution (*right*), there is an abrupt decline of current followed by a more gradual decay. Owing to the several seconds that are needed to obtain a full exchange of the fluid bathing the oocyte, we have not analyzed the kinetics of this process. D, Recovery of responsiveness to kainate after metal ion blockade. Only partial recovery of the original kainate response is observed in oocytes exposed to 1 μM Hg^{2+} and then washed for 10 min with normal frog Ringer solution. Full recovery is seen after a similar protocol using Cd^{2+} (1 mM).

if the group IIb ions are added to the bathing solution before or concomitantly with kainate (results not shown). Of more importance is the fact that Hg^{2+} (but not Cd^{2+} or Zn^{2+}) inhibits the kainate-triggered current even when the Hg^{2+}-containing bath solution is washed out before exposure of the oocytes to agonist (Fig. 2B). To minimize any problems with obtaining a satisfactory removal of bath Hg^{2+}, we conducted these trials using relatively low concentrations of Hg^{2+}. As shown (Fig. 2B), even with a 2-min wash the response of an oocyte to a second challenge with kainate (following a 70-sec exposure to Hg^{2+}) results in a 52% decline of current. In six such experiments, the mean reduction of the current in the second trial was 51 ± 2%. This contrasts with the control situation (no Hg^{2+} exposure), where the second challenge with kainate is 97 ± 2% of the first trial (n = 8). These results imply that the principal Hg^{2+} binding site of the kainate receptor-channel complex is exposed even when the agonist-gated channel is closed. Similar results were obtained by Kiskin *et al.* (14).

The relatively low concentration of Hg^{2+} that is required to block completely the kainate-evoked current of these oocytes (Fig. 2A) prompted us to focus on the mechanism of this action and to compare it with the depressant effects (Fig. 2A) of Cd^{2+} and Zn^{2+}. One factor that hampered these experiments is that Hg^{2+} (but not Cd^{2+} or Zn^{2+} up to 2 mM) can cause an *irreversible* decline of the membrane resistance of oocytes. This irreversible decline of membrane resistance yields an *apparent* inward current. The threshold for these effects varies among oocytes within the range of 0.25 to 2.5 μM Hg^{2+}. Oocytes exhibiting such behavior (in which Hg^{2+} appeared erroneously to enhance rather than inhibit the kainate-activated current) were discarded.

In view of the potential for irreversible toxic effects of Hg^{2+}, all experiments were confined to cells that exhibited no loss of membrane resistance up to 1 μM Hg^{2+}. Under these circumstances, the inhibition by Hg^{2+} of the kainate-evoked current often appeared to be biphasic. This effect was most evident with concentrations of Hg^{2+} that did not fully block the inward current (Fig. 2C; see also Fig. 2B). A rapid reduction (about 15%) of current amplitude was followed by a more gradual decline over a period of 5 min (Fig. 2C). To minimize the possibility that this slower decay of current was due to receptor desensitization, we used oocytes in which stable reproducible responses to kainate could be elicited. As shown in Fig. 2C, *left*, the kainate-evoked currents in oocytes with stable resting potentials exhibit almost no desensitization over periods of 5 min. Thus, the fast and slow components of Hg^{2+} action imply that there may be more than one binding site for this metal ion. However, we cannot exclude the possibility that there are multiple sequelae of the binding of Hg^{2+} to a single site. A combination of ligand binding studies, single-channel investigations, and higher resolution kinetic experiments (which are hindered by the large diameter of the oocyte) should resolve this issue.

In contrast to the biphasic action of Hg^{2+}, the block produced by Cd^{2+} or Zn^{2+} was monophasic (data not shown). These agents caused a rapid decline of the kainate-evoked current (as seen in Fig. 2A) with no apparent slower component like that seen with Hg^{2+} (Fig. 2C).

The recovery of the kainate response after inhibition by Hg^{2+} is slow and incomplete. For example (Fig. 2D), after 10 min of washing with frog Ringer solution, less than 30% of the agonist-induced response returns in an oocyte that had been exposed to Hg^{2+} (1 μM). The block produced by either Cd^{2+} or Zn^{2+} is more readily reversible than the Hg^{2+} block (Fig. 2D). In general, we observe a full recovery of the response to kainate of oocytes exposed to 1 mM Zn^{2+} or 1 mM Cd^{2+} and then washed with frog Ringer solution (the Cd^{2+} example is shown in Fig. 2D). However, we do not see full recovery of the kainate current after blockade with Hg^{2+}. Even extensive washing (30 min) of an oocyte exposed to 1 μM Hg^{2+} fails to restore the kainate response to its original amplitude.

The biphasic onset of the Hg^{2+} block (Fig. 2C) and the largely irreversible nature of this antagonism (Fig. 2D) compelled us to choose the following protocol to assess the inhibitory potency of Hg^{2+} relative to Cd^{2+} and Zn^{2+}. We sequentially applied to

individual oocytes solutions with increasing concentrations of metal ion and a fixed concentration of kainate (1 mM). We measured the percentage of reduction of the original response (obtained with kainate alone) 20 sec after a new concentration of metal ion was applied. This paradigm ignores the slower decline of kainate-evoked current seen with Hg^{2+} (Fig. 2B) but gives a measure of the relative IC_{50} for inducing the rapid phase of block. This protocol will underestimate the level of Hg^{2+}-mediated block at lower Hg^{2+} concentrations and it should overestimate the percentage of inhibition at higher Hg^{2+} levels, which are applied after some of the slow phase of block has developed. In spite of this constraint, the data show that Hg^{2+} produces a 50% block at about 70 nM, whereas the IC_{50} for Cd^{2+} is 0.16 mM and for Zn^{2+} it is 0.22 mM (Fig. 3A). The data for Cd^{2+} and Zn^{2+} are comparable to values obtained in studies of rat brain kainate receptors expressed in oocytes (15).

The data of Fig. 3B show that Hg^{2+}, Zn^{2+}, and Cd^{2+} block the kainate-evoked current in a noncompetitive manner. There is a depression by these ions of the peak response evoked by kainate, but there is very little lateral shift of the concentration-response curve. Moreover, these ions block in a voltage-independent fashion (Fig. 3C). With Hg^{2+} in the presence of kainate, we see no appreciable change either in the shape of the current-voltage relation or in the reversal potential (Fig. 3C). Similar results are obtained with Zn^{2+} and Cd^{2+} (data not shown). Very weak voltage dependence is associated with Cd^{2+} and Zn^{2+} antagonism of kainate currents of oocytes injected with rat brain mRNA (15).

Action of thiol-modifying reagents. The features of the Hg^{2+}-mediated depression of kainate responses (rapid, noncompetitive, voltage-independent onset and a slow and incomplete recovery) suggested that this metal might be binding to a functionally important thiol group of this receptor-channel complex. This site would be crucial for ion permeation, but the lack of demonstrable voltage dependence implies that it is not directly in the ion permeation pathway. Corroborative evidence for this hypothesis was obtained by showing that other reagents that modify protein sulfhydryl groups (27, 28) can also impair kainate-triggered currents of RNA-injected oocytes (Fig. 4). Examples of the inhibition produced by three of these reagents are given in Fig. 4. Iodoacetamide, dithiobisnitrobenzoic acid, and PCMB produce significant reductions of the kainate-evoked current, with threshold concentrations between 0.01 and 0.2 mM. We also found that mersalyl (0.1 mM) blocks more than 75% of the current evoked by 1 mM kainate, but *N*-ethylmaleimide (1 mM) causes less than a 25% reduction of the kainate response (data not shown). Indeed, the IC_{50} (approximately 50 μM) for the two mercury-containing thiol reagents was 1 order of magnitude lower than the IC_{50} for compounds like iodoacetamide or dithiobisnitrobenzoic acid. Moreover, the inhibition produced by the Hg-containing organic thiol reagents was not reversed by 10 min of washing with normal frog Ringer solution. These data are consistent with the involvement of one or more thiol groups in the regulation of ion flux associated with this class of amino acid receptor.

Reversal of Hg^{2+} effects. Kiskin and colleagues (14) reported that cysteine or glutathione can reverse the Hg^{2+} inhibition of kainate-evoked currents in rat hippocampal neurons.

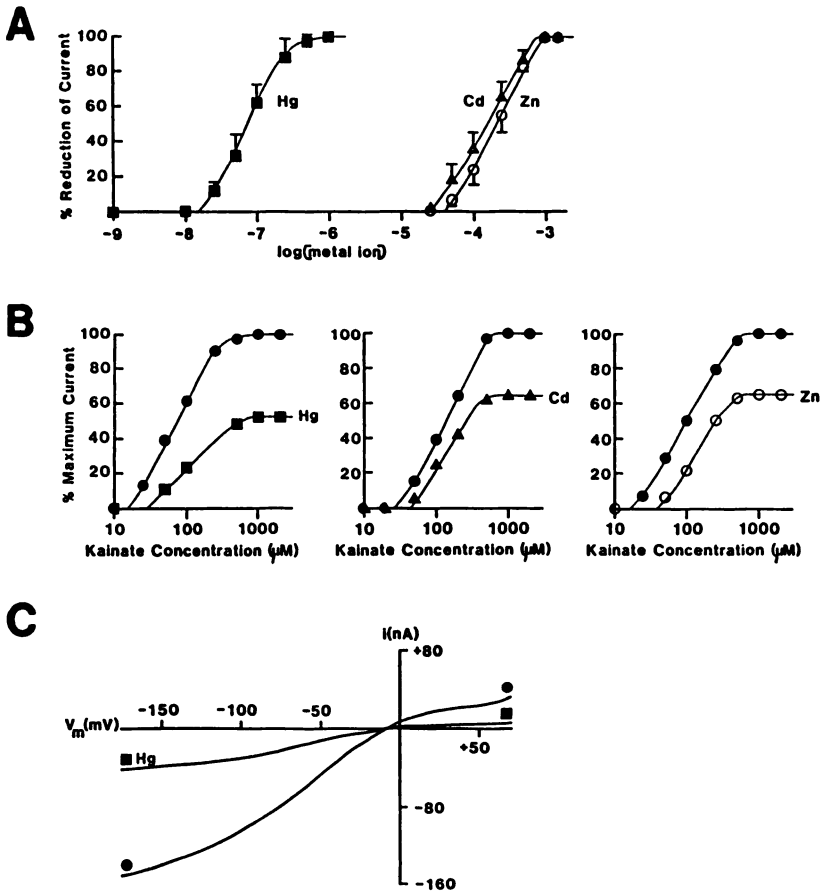

Fig. 3. A, Concentration-response curves for the blockade of kainate-triggered currents by Hg^{2+}, Cd^{2+}, and Zn^{2+}. For each series of measurements, a kainate (1 mM) response was elicited in RNA-injected oocytes and then the kainate-containing solution with increasing concentrations of metal ion was applied to the oocyte. The percentage of decline of the kainate-triggered current (relative to that total amount of current that could be blocked) was measured 20 sec after exposure to a new concentration of metal ion. The results plotted are the mean ± standard deviation of four oocytes from two different frogs. Note that this protocol does not take into account the slower decline of current amplitude that is triggered by these metal ions (see Fig. 2). B, Effect of increasing kainate concentrations on the block produced by Hg^{2+}, Cd^{2+}, and Zn^{2+}. Individual RNA-injected oocytes were exposed to increasing concentrations of kainate in the absence and then the presence of a fixed concentration of Hg^{2+} (50 nM), Cd^{2+} (0.1 mM), or Zn^{2+} (0.1 mM). The results presented are representative data from single oocytes. Similar results were obtained in two other experiments. C, Current-voltage relation of an RNA-injected oocyte with and without Hg. Conditions were as in Fig. C, but Hg^{2+} (0.1 μM) was present. ●, Control curve; ■, in the presence of Hg^{2+}. This example is typical of results of four such experiments.

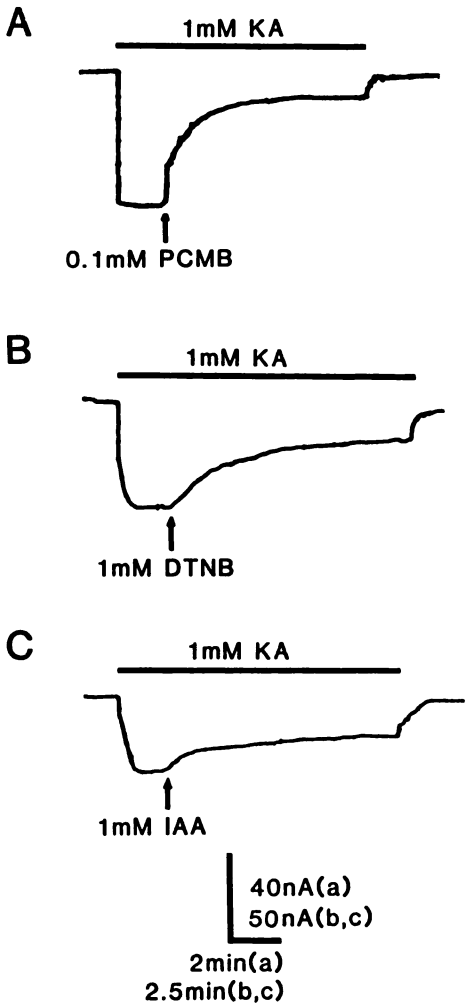

Fig. 4. Blockade of kainate-evoked currents by different thiol reagents. After obtaining stable responses to kainate (1 mM) in RNA-injected oocytes, the bath solution was switched to one containing kainate (1 mM) plus the indicated concentration of thiol reagent. *DTNB*, dithiobisnitrobenzoic acid; *IAA*, iodoacetamide.

We obtained similar findings with the observation that either cysteine (1 mM) or dithiothreitol (1 mM) greatly accelerates the recovery of kainate-triggered currents of oocytes exposed to Hg^{2+} (Fig. 5). We have not obtained full recovery of the kainate-triggered current under these conditions. But, in experiments with 1 mM cysteine, more than 85% (n = 6) of the original current returned, in contrast with the persistent block observed when no thiol-reducing agent is added (Fig. 2).

Interaction of CNQX and Hg^{2+}. Owing to the high affinity and relative irreversibility of the Hg^{2+} blockade of kainate currents, our results cannot rule out the possibility that Hg^{2+} binds at or near the kainate binding site to prevent the agonist from binding. Because CNQX is a competitive antagonist of kainate (Fig. 1C) with a considerably higher affinity for the receptor, we assessed whether CNQX might, in stoichiometric excess, protect the kainate response from inhibition by Hg^{2+}. The experiments were identical to those of Fig. 2B, except that CNQX (20 μM) was present for 30 sec before, during, and for 30 sec after exposure to Hg^{2+}. Under these circumstances, we saw no diminution in the blocking potency of Hg^{2+} relative to trials without CNQX. This result argues against a significant binding of Hg^{2+} to the site(s) occupied by agonist or antagonist.

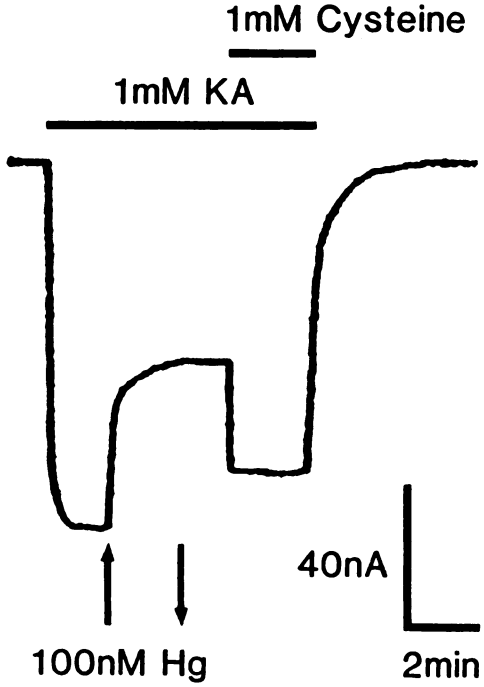

Fig. 5. Cysteine accelerates the recovery of kainate-evoked currents after Hg^{2+} inhibition. After the kainate response of an oocyte was inhibited with 0.1 μM Hg^{2+}, cysteine (1 mM) was included in the bath solution as indicated. The response of the oocyte to a second application of kainate had recovered to 86% of the original.

Discussion

The data assembled in this report indicate that Hg^{2+} impairs the function of the kainate receptor-channel complex by binding to a thiol group that is neither intimately associated with the agonist binding site nor an integral part of the ion channel. Rather, it appears likely that Hg^{2+} binds to a site that is important for the coupling of agonist occupancy to the maintenance of the channel in the open conformation. These conclusions about the nature of the Hg^{2+} binding site and its function are based on the following results. The stability constants for Hg-sulfhydryl complexes are many orders of magnitude higher than comparable constants for Zn or Cd complexes (27–29). Thus, the high potency of the block of kainate responses by Hg^{2+}, relative to Cd^{2+} and Zn^{2+}, is consistent with an action at a thiol group. So, too, are the observations of the slow and incomplete washout of Hg^{2+} effects and the reversibility of the Hg^{2+}-mediated inhibition of kainate currents by thiol compounds like cysteine and dithiothreitol. Moreover, thiol-oxidizing or -alkylating reagents, particularly those incorporating Hg in their structure (e.g., PCMB) are effective blockers of kainate-gated currents.

Evidence that Hg^{2+} is not binding in or close to the kainate-regulated cation channel comes from the observation of the lack of voltage dependence of the Hg^{2+} effect, plus the fact that Hg^{2+} remains a potent antagonist even when the channels are opened by a saturating concentration of kainate. If Hg^{2+} were plugging the ion-conducting pore, one would anticipate a degree of voltage dependence of the block, along with a capacity for the premeant cations to impair Hg^{2+} binding in the pore. No evidence for either of these scenarios was obtained.

Concentration-response curves show little change in the affinity of kainate for its receptor in the presence of Hg. Rather,

there is a depression in the maximum response that is typical of noncompetitive antagonists. Nevertheless, in view of the greatly differing affinities of Hg^{2+} and kainate for their respective binding sites, we tested the interaction of Hg^{2+} and CNQX, which is a competitive kainate receptor antagonist with an IC_{50} more comparable to that of Hg^{2+}. Our rationale is that if Hg^{2+} were to bind at or near the kainate binding domain, CNQX, with its higher affinity for this site, might antagonize the persistent block due to Hg^{2+}. Because CNQX failed to do so, we conclude that Hg^{2+} binds to a site distinct from that of agonists and antagonists.

Our results are also consistent with the possibility that Cd^{2+} and Zn^{2+} inhibit the kainate-gated currents via the same thiol to which Hg^{2+} binds. However, we cannot exclude the explanation that there is an independent anionic site, such as that proposed by Randle and colleagues (15), which can modulate the ion flux of this receptor-channel complex. One way to merge the thiol group/anionic site hypotheses is to suggest that it is a S^- site that binds the heavy metals and thiol reagents. Although there are attractive features of this proposal, we note that the negatively charged thiol-modifying reagents (e.g., dithiobisnitrobenzoic acid or PCMB) were, if anything, more potent blockers of kainate-gated currents than were neutral thiol reagents like iodoacetamide (a feature that would not be expected if electrostatic forces govern the affinity of this site for ligands). Hence, the possibility remains that there are both an anionic site and a thiol group that regulate the ion flux of the kainate receptor-channel complex.

With only minor differences, our results confirm those obtained by Kiskin and collaborators (14) in their study of sulfhydryl reagent actions on kainate-evoked currents of rat hippocampal neurons. In their experiments (14), Zn^{2+} was a somewhat weaker antagonist (effective in the 5–10 mM range), whereas both our results and those of Randle and coworkers (15) indicate an IC_{50} below 1 mM. We also observed an apparent slow component of the Hg^{2+}-mediated block of kainate-evoked currents, whereas Kiskin *et al.* (14) documented a single-exponential decay under similar conditions. However, their data did not contain examples of longer term exposures to Hg^{2+}, which may be necessary to detect a slow component of block. Regardless, the nature of the interaction responsible for this slow component of block remains to be illuminated by higher resolution kinetic experiments that are not practical using bath-perfused oocytes. Otherwise, the close agreement between our data and those of Kiskin *et al.* (14) supports the contention that sulfhydryl groups are important for kainate receptor function.

Hg^{2+} was essentially equipotent in its blockade of kainate-gated currents in oocytes and rat hippocampal neurons (14). This convergence of our data with earlier results (14) is strong support for the proposal that the Hg^{2+} actions in these two systems are exerted solely on the kainate subclass of glutamate receptor. Moreover, the fact that neither NMDA nor quisqualate, by themselves, elicits any appreciable current in the oocytes injected with the one preparation of human brain RNA we employed also argues against a role for other glutamate receptor subtypes in these effects. However, in preliminary experiments, we made the following observations regarding Hg^{2+} effects on NMDA- or quisqualate-triggered currents. The slow, oscillating, multicomponent response evoked by quisqualate in oocytes injected with human or rat brain mRNA (e.g., see Refs. 18, 21, and 22) is inhibited by Hg^{2+}.[1] We are attempting to obtain more-quantitative data regarding these effects of Hg^{2+}. In contrast, NMDA responses have been unaffected, and in some instances even potentiated, by submicromolar levels of Hg^{2+}.[1] It will be important to examine further these effects of Hg^{2+}, particularly in view of the ligand-binding data recently reported by Terramani and colleagues (16). They assessed the effects of a variety of sulfhydryl-modifying reagents on the binding of ligands to different glutamate receptor subtypes of rat synaptic membranes (16). In concert with our results and those of Kiskin *et al.* (14), they saw little effect of PCMB on kainate binding. However, PCMB and Hg^{2+} increased [^{3}H]AMPA binding to quisqualate receptor sites, principally by increasing the B_{max} of a high affinity component and by lowering the K_m of a low affinity component (16). These agents also increased slightly the binding of [^{3}H]glutamate to NMDA sites (16). Future investigations will be necessary to correlate the physiological data with these ligand-binding results.

The peripheral nicotinic acetylcholine receptor, a prototype of the "excitatory" neurotransmitter receptor-channel complex, has a disulfide bond located near the acetylcholine binding site and one or more sulfhydryl groups that modulate ion translocation (30, 31). However, relative to the concentrations found to be effective against kainate responses, considerably higher levels of thiol-modifying reagents are needed to inhibit ion flux associated with the nicotinic acetylcholine receptor (31). The relatively high sensitivity of kainate receptors to block by certain thiol compounds, Hg^{2+} in particular, suggests that some of the neurological manifestations of mercury poisoning (e.g., erethism and ataxic intention tremor; see Refs. 32, 33) may be attributable to actions on kainate receptors. Finally, because Hg^{2+} is well known for its use in the X-ray analysis of protein structure (34, 35), it may be a valuable adjunct in the further characterization of the structure and function of the kainate receptor-channel complex.

Acknowledgments

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