Block of open channels of recombinant AMPA receptors and native AMPA/kainate receptors by adamantane derivatives

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1. The effects of two adamantane derivatives, 1-trimethylammonio-5-(1-adamantane-methyl-ammoniopentane dibromide) (IEM-1460) and 1-ammonio-5-(1-adamantane-methylammonio-pentane dibromide) (IEM-1754) on kainate-induced currents were studied in *Xenopus* oocytes expressing recombinant ionotropic glutamate receptors and in freshly isolated neurones from rat hippocampal slices.

2. The adamantane derivatives caused use- and voltage-dependent block of open channels of recombinant AMPA receptors. This antagonism was dependent on receptor subunit composition; channels gated by recombinant, homomeric GluR1 and GluR3 receptors exhibited a higher sensitivity to block than those gated by receptors containing edited GluR2 subunits. In the former cases, IEM-1460 had an IC_{50} of 1·6 μM at a holding potential (V_h) of −80 mV and IEM-1754 was 3·8 times less potent than IEM-1460. In contrast, 100 μM IEM-1460 inhibited responses to 100 μM kainate of receptors containing edited GluR2 subunits by only 7·8 ± 2·4% ($n = 5$ oocytes) at a V_h of −80 mV.

3. Native AMPA/kainate receptors in isolated hippocampal cells were inhibited by adamantane derivatives in a use- and voltage-dependent manner. This antagonism was dependent on cell type: pyramidal neurones were less sensitive to IEM-1460 ($IC_{50} = 1617$ μM at $V_h = -80$ mV) than interneurones ($IC_{50} = 1{\cdot}6$ μM at $V_h = -80$ mV). IEM-1460 and IEM-1754 were equipotent when applied to pyramidal neurones, but IEM-1754 was less potent (~3 times) than IEM-1460 when applied to interneurones.

4. It is concluded that the presence of the edited GluR2 subunit in recombinant AMPA receptors and native AMPA/kainate receptors inhibits channel block by organic cations and that adamantane derivatives are potentially valuable tools for identifying classes of AMPA/kainate receptors and their roles in synaptic transmission.

Mammalian ionotropic glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type are multimeric proteins, the functional properties of which depend on their subunit (GluR1–4) composition (Hollmann & Heinemann, 1994; Jonas & Burnashev, 1995; Ozawa & Rossier, 1996). Receptors assembled from recombinant GluR1, GluR3 and GluR4 subunits gate channels that are highly permeable to Ca^{2+}, whereas those that include one or more edited GluR2 subunits gate channels of low Ca^{2+} permeability (Hume, Dingledine & Heinemann, 1991; Burnashev, Monyer, Seeburg & Sakmann, 1992). Although native AMPA/kainate receptors gating channels with high Ca^{2+} permeability have been found only sparingly in different parts of the mammalian brain (Iino, Ozawa & Tsuzuki, 1990; Geiger *et al.* 1995; Koh, Geiger, Jonas & Sakmann, 1995; Gotz, Kraushaar, Geiger, Lubke, Berger & Jonas, 1997), immunocytochemical analysis and analysis of mRNA of single neurones has suggested a good correlation between the expression of edited GluR2 subunits and Ca^{2+} permeability (Bochet *et al.* 1994; Jonas, Racca, Sakmann, Seeburg & Monyer, 1994; Geiger *et al.* 1995).

There are reasons to believe that the Ca^{2+} permeability of AMPA receptor channels and their block by organic cations are associated with similar structural features of the putative membrane segments of GluR1–4 subunits. The

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polycation argiotoxin-636 (ArgTX-636) blocks channels of homomeric GluR1, GluR3 and GluR4 receptors expressed in *Xenopus* oocytes, whereas it is only a weak blocker of channels gated by heteromeric receptors containing edited GluR2 subunit(s) (Blaschke, Keller, Rivosecchi, Hollmann, Heinemann & Konnerth, 1993; Brackley, Bell, Choi, Nakanishi & Usherwood, 1993; Herlitze *et al.* 1993). Joro spider toxin (JSTX) blocks Ca^{2+}-permeable AMPA/kainate channels in native neurones (Tsubokawa, Oguro, Masuzawa, Nakaima & Kawai, 1995; Iino, Koike, Isa & Ozawa, 1996). The correlation between high Ca^{2+} permeability and channel block by organic cations also applies to quisqualate-sensitive receptors of insect muscle. These ionotropic glutamate receptors gate Ca^{2+}-permeable channels that are readily blocked by polyamine amides such as philanthotoxin-433 (Clark *et al.* 1982), philanthotoxin-343 (PhTX-343; Sudan *et al.* 1995) and ArgTX-636 (Kerry, Ramsey, Sansom & Usherwood, 1988; Antonov, Dudel, Franke & Hatt, 1989), and by biscationic adamantane derivatives (Magazanik, Antonov & Gmiro, 1984). In cultured rat cortical neurones, the same derivatives of adamantane only weakly antagonize currents induced by kainate, although they are highly potent non-competitive antagonists of responses to *N*-methyl-D-aspartate (NMDA) (Antonov, Johnson, Lukomskaya, Potapyeva, Gmiro & Magazanik, 1995). Such differences, which also characterize the polyamine amides (Green, Nakanishi & Usherwood, 1996), may result from the predominance in these cells of heteromeric AMPA receptors containing edited GluR2 subunits. The results reported herein provide further support for this assumption. It will be shown that cells freshly isolated from rat hippocampus differ in their sensitivity to two adamantane derivatives: kainate-activated whole-cell currents recorded from non-pyramidal cells are more sensitive to these compounds than those recorded from pyramidal cells. Such observations are consistent with published data on the Ca^{2+} permeability of AMPA/kainate receptor channels in these two classes of neurone (Bochet *et al.* 1994; Jonas *et al.* 1994; Geiger *et al.* 1995). It will be shown also that channels gated by recombinant, homomeric GluR1 and GluR3 receptors expressed in oocytes exhibit a high sensitivity to channel block by the two adamantane derivatives, whereas much higher concentrations of these drugs are required to cause equivalent antagonism of recombinant GluR1 plus edited GluR2 receptors and GluR3 plus edited GluR2 receptors. A preliminary account of this work has already been published (Magazanik, Buldakova, Samoilova, Mellor & Usherwood, 1997).

METHODS

Expression of recombinant AMPA receptors in oocytes

mRNAs were transcribed from the non-NMDA receptor subunit clones GluR1, edited GluR2 and GluR3. Oocytes were obtained from adult female *Xenopus laevis* under anaesthesia (0·2% 3-aminobenzoic acid solution; Sigma) and manually separated. Each oocyte was injected with 50 nl (1 ng nl^{-1}) of mRNA for expression of homo-oligomeric receptors or 25 nl of each mRNA for hetero-oligomeric receptor expression. The oocytes were incubated under sterile conditions at 18 °C in Barth's solution (Brackley *et al.* 1993).

Electrophysiological recording from oocytes

Four to seven days after injection of mRNA, oocytes were placed in a Silicone tube of 2 mm diameter and continuously perfused with saline (mM: 120 NaCl, 2 KCl, 1·8 $CaCl_2$ and 9·5 Hepes; pH 7·4) at 22–24 °C. Currents elicited by 100 μM kainate (Sigma) were measured using a conventional two-microelectrode voltage clamp (Axoclamp 2A, Axon Instruments). Each application of kainate lasted 20–40 s. When the current induced by this agonist reached a steady state, the saline flow was switched to a solution containing the same concentration of kainate plus a given concentration of IEM-1460 or IEM-1754. Again, on reaching a steady state, the saline flow was returned to a solution containing kainate alone when recovery of the response to the agonist was recorded. This procedure was repeated several times, at not less than 5 min intervals, with different concentrations of the adamantane derivative. It was possible to conduct several experiments on a single oocyte. Unless otherwise noted, membrane currents were measured at a holding potential (V_h) of −80 mV. The voltage dependence of antagonism was estimated by ramping the membrane potential from −140 mV to +40 mV, the duration of the ramp being 20 s. Voltage and current responses were recorded on magnetic tape and post-analysed using in-house software (R. L. Ramsey).

Preparation of freshly isolated cells

Cells were isolated from transverse slices of hippocampus taken from 15- to 30-day-old Wistar rats. Briefly, after urethane anaesthesia and decapitation the brain was removed, and 200–500 μm thick slices were cut with a razor blade. The slices were incubated at 30–32 °C in a solution containing (mM): 124 NaCl, 5 KCl, 1·3 $CaCl_2$, 1·5 $MgCl_2$, 20 $NaHCO_3$, 1·24 NaH_2PO_4 and 10 D-glucose. The medium was gassed with 95% O_2–5% CO_2 (pH 7·4–7·5). After 2–9 h incubation, the slices were transferred to the recording chamber. Cells were freed from a slice by vibrodissociation at 70–120 Hz (without enzymatic treatment of the tissue) (Vorobjev, 1991). All manipulations and cell identifications were performed using an inverted microscope.

Whole-cell recordings

Experiments were undertaken at 22–24 °C. The following extracellular solution was used for dissociation of cells and superperfusion of isolated cells (mM): 143 NaCl, 5 KCl, 2·5 $CaCl_2$, 10 D-glucose and 10 Hepes–NaOH; pH 7·4. Currents elicited by kainate were recorded in the whole-cell patch-clamp configuration, using patch pipettes, with resistances of 3–5 MΩ. Recordings were made using an Axopatch 200A amplifier (Axon Instruments). Patch pipettes were filled with solution containing (mM): 100 CsF, 40 CsCl, 5 NaCl, 0·5 $CaCl_2$, 5 EGTA and 10 Hepes–CsOH; pH 7·2. Kainate, either alone or in combination with an adamantane derivative, was applied through a thin (0·2 mm diameter) glass capillary located in a glass tube (1·5 mm diameter) through which the control solution constantly flowed. The patch pipette, with an isolated neurone in the whole-cell recording configuration, was placed initially in the tube so that the cell was perfused by the control solution. It was then rapidly and temporarily exposed, under computer control, to the thin stream of solution containing either agonist or agonist plus antagonist. This method of drug application was described by Vorobjev, Sharonova & Haas (1996). The following experimental protocol was employed. Every 20 s, pulses (1 s duration) of kainate or kainate plus antagonist were applied to a cell. The control of V_h, application of agonist and

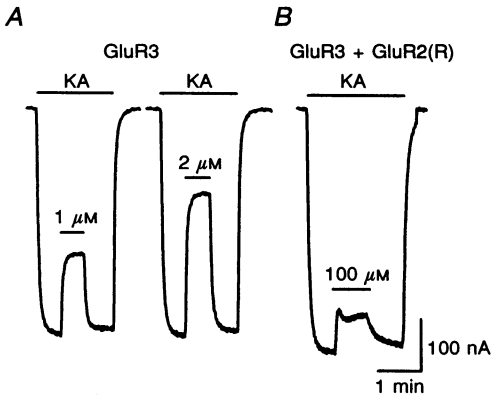


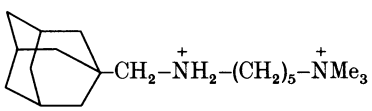
Figure 1. Influence of the edited GluR2 subunit on antagonism by IEM-1460

Antagonism by IEM-1460 of currents induced in *Xenopus* oocytes by 100 μM kainate (KA). *A*, an oocyte injected 7 days previously with GluR3 mRNA. *B*, an oocyte co-injected 5 days previously with GluR3 plus edited GluR2 mRNAs. Long bars, application of 100 μM kainate; short bars, co-application of kainate and 1, 2 and 100 μM IEM-1460. IEM-1460 was more potent in *A* than in *B*. $V_h = -80$ mV.

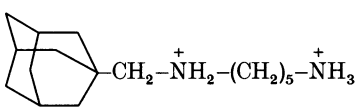
agonist plus antagonist, and data acquisition were achieved using a PC and in-house software (PATCH3, D. B. Tikhonov, Sechenov Institute of Evolutionary Physiology and Biochemistry, St Petersburg).

Drugs

Kainate was purchased from Sigma. Adamantane derivatives 1-trimethylammonio-5-(1-adamantane-methylammoniopentane dibromide) (IEM-1460) and 1-ammonio-5-(1-adamantane-methylammoniopentane dibromide) (IEM-1754) were synthesized in the Institute of Experimental Medicine, Russian Academy of Medical Sciences. PhTX-343 was supplied by K. Nakanishi, Department of Chemistry, Columbia University, New York.



IEM-1460



IEM-1754

Data analysis and statistics

The data were expressed as means ± S.E.M. from n observations (oocytes or isolated cells). The significance of differences between means was calculated using one-way ANOVA. A P value of < 0.05 was considered to indicate a significant difference between two means.

IC_{50} values were obtained by fitting the data using the formula:

$$\text{Percentage inhibition} = 100\%/[1 + ([D]/IC_{50})^s],$$

where [D] is the antagonist concentration, IC_{50} is the antagonist concentration producing 50% inhibition and s is the slope of the concentration–inhibition curve.

RESULTS

Antagonism by adamantane derivatives of homomeric and heteromeric AMPA receptors expressed in oocytes

Inhibition of kainate-induced currents by IEM-1460. Sustained inward currents of 60–450 nA were obtained at $V_h = -80$ mV when 100 μM kainate was applied to oocytes expressing homomeric GluR1 or GluR3 receptors. Similar currents were recorded from oocytes expressing heteromeric receptors containing combinations of either GluR1 or GluR3 with the edited GluR2 subunit. IEM-1460 alone did not elicit currents, but it inhibited currents elicited by kainate (Fig. 1). Figure 2 illustrates a concentration–inhibition relationship for IEM-1460 on oocytes expressing homomeric GluR1 or GluR3 receptors (pooled data); the IC_{50} is 1·6 μM at −80 mV. Figure 1 shows typical results obtained from oocytes injected with mRNAs for either GluR3 or GluR3 plus edited GluR2. IEM-1460 was more potent as an antagonist of responses to kainate of homomeric GluR3 than of heteromeric GluR3 plus edited GluR2. In the latter

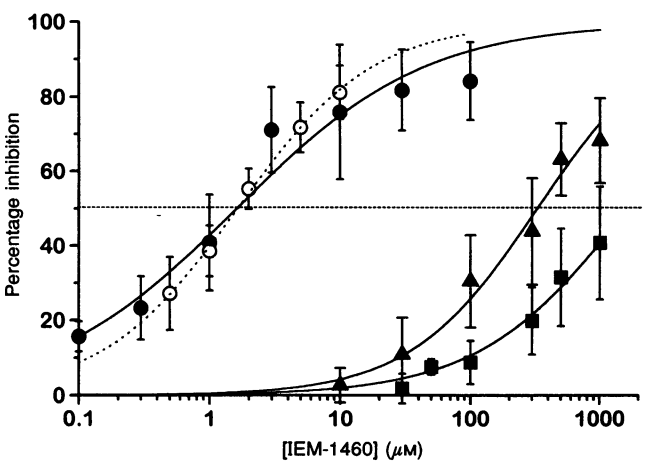


Figure 2. Antagonism of recombinant and native ionotropic glutamate receptors

Concentration curves for inhibition by IEM-1460 of responses to 100 μM kainate. ○, oocytes injected with mRNAs of either GluR1 or GluR3 (pooled data from 10 oocytes), $V_h = -80$ mV. ●, ▲, ■, freshly isolated rat hippocampal cells. ●, cells with high sensitivity to antagonist; 3–8 observations from 19 cells. ▲, cells with intermediate sensitivity to antagonist; 3–23 observations from 42 cells. ■, cells with low sensitivity to antagonist; 5–57 observations from 73 cells. $V_h = -80$ mV. Bars are S.E.M. Concentration–inhibition curves were fitted logistically (see Methods) and drawn by continuous lines for isolated cells and by a dotted line for oocytes.

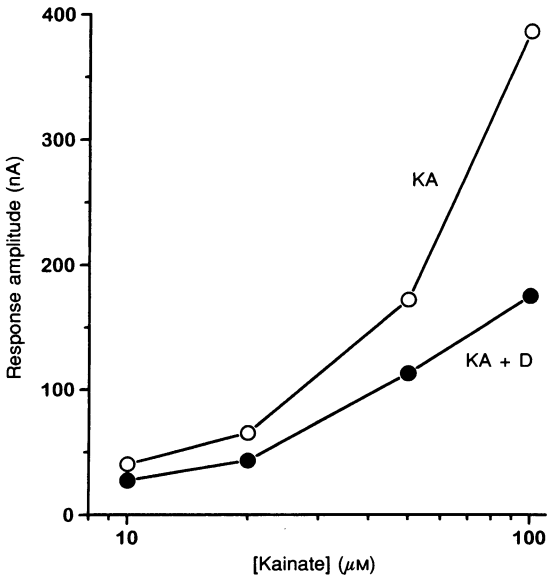


Figure 3. Non-competitive antagonism by IEM-1460 of responses to kainate of recombinant homomeric GluR1 receptors

Concentration–response relationships for kainate (KA) in the absence (○) and in the presence (●) of 1 μM IEM-1460 (KA + D). $V_h = -80$ mV.

case 100 μM IEM-1460 inhibited responses to 100 μM kainate by only 7·8 ± 2·4% ($n = 5$ oocytes) at a V_h of −80 mV. According to the curve presented in Fig. 2, an equivalent antagonism of homomeric GluR3 receptors would be induced by 0·1 μM IEM-1460. Similar comparative data (not shown) were obtained with GluR1 and GluR1 plus edited GluR2.

Mechanism of antagonism. The representative data illustrated in Fig. 3 suggest that IEM-1460 non-competitively inhibited the responses of homomeric GluR3 receptors to kainate, i.e. the percentage antagonism by a given concentration of IEM-1460 did not decrease when the concentration of kainate was raised. The antagonism induced by IEM-1460 probably does not involve closed channel block, because a 10 min pre-treatment of an oocyte expressing homomeric GluR3 receptors with 1 μM of this compound did not affect the response to 100 μM kainate when the agonist was applied immediately after a 5 s intensive washout of the antagonist (Fig. 4*A*, 1 and 2). However, if recovery from closed channel block was fast then it would be missed by this procedure. Co-application of the same concentrations of kainate and IEM-1460 induced an inward current, the peak of which was 85% of that elicited by kainate alone (Fig. 4*A*, 3). During a further 90 s co-application of 100 μM kainate and 1 μM IEM-1460, the response fell to 50%. These results suggest that IEM-1460 is a blocker of the open channel gated by homomeric GluR1 and GluR3 receptors, and the equilibrium between blocked and unblocked channels was attained slowly.

Use and voltage dependence of antagonism by IEM-1460. Inhibition by IEM-1460 of the responses of homomeric GluR1 and GluR3 receptors to kainate persisted

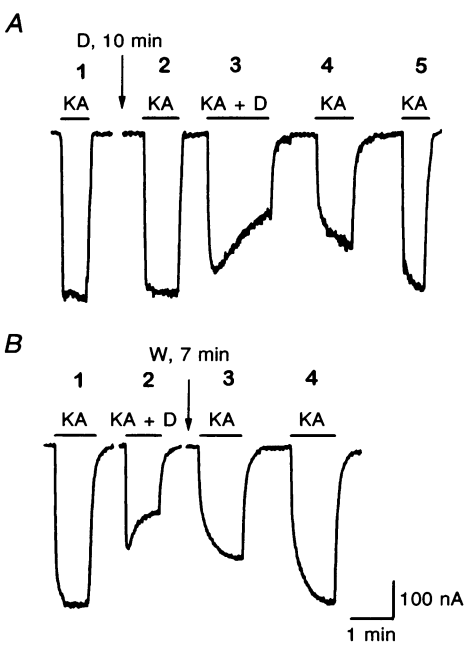


Figure 4. Use dependence of antagonism by IEM-1460

Representative responses to application of 100 μM kainate (KA) alone, 2 μM IEM-1460 (D) alone, and KA (100 μM) plus D (2 μM) in oocytes injected with GluR1 mRNA. *A*, 1, response to kainate application alone. After a 5 s wash with saline, IEM-1460 was applied alone for 10 min (arrow). 2, response to application of kainate alone immediately after removal of IEM-1460. Response to co-application of kainate and IEM-1460 (3), followed by responses to kainate 2 min (4) and 4 min (5) after co-application of kainate and IEM-1460. Note that the 10 min application of IEM-1460 alone did not inhibit the subsequent response to kainate (2), but that during co-application of kainate and IEM-1460 a progressive reduction of the kainate-induced current occurred (3). *B*, 1, inward current obtained in response to application of kainate alone. 2, response to co-application of kainate and IEM-1460. After a 7 min wash (W) in saline (arrow) a response to kainate alone was elicited (3) followed by a further response to kainate alone 3 min later (4). Note that the rate of recovery of the response to kainate after antagonism induced by IEM-1460 was accelerated by subsequent applications of kainate. $V_h = -80$ mV for all traces.

Table 1. Inhibitory effects of IEM-1460 and IEM-1754 on kainate (100 μM) responses in oocytes and isolated hippocampal cells at different holding potentials (V_h)

Preparation	Drug	Concentration (μM)	Percentage inhibition $V_h = -40$ mV	$V_h = -80$ mV	$V_h = -120$ mV
Recombinant homomeric GluR1 or GluR3 receptors in oocytes	IEM-1460	1·0	9·1 ± 4·3 (4)	16·5 ± 3·2 (4)**	41·4 ± 7·8 (4)**
	IEM-1754	5·0	15·6 ± 12·4 (4)	46·1 ± 7·7 (8)**	61·0 ± 7·6 (3)*
Low-sensitivity neurones	IEM-1460	100·0	8·1 ± 6·0 (7)	8·8 ± 5·8 (57)†	21·2 ± 10·1 (26)**
	IEM-1754	100·0	11·0 ± 5·4 (9)	11·7 ± 5·3 (25)†	26·4 ± 7·1 (9)**
Intermediate-sensitivity neurones	IEM-1460	100·0	11·7 ± 9·5 (7)	30·5 ± 12·4 (23)*	52·8 ± 11·0 (27)**
	IEM-1754	100·0	14·3 ± 8·8 (4)	29·7 ± 12·5 (9)*	45·8 ± 8·0 (6)*
High-sensitivity neurones	IEM-1460	3·0	54·0 ± 6·3 (3)	71·1 ± 11·5 (7)*	57·7 ± 8·3 (3)†
	IEM-1754	3·0	18·3 ± 5·0 (3)	24·0 ± 3·0 (5)†	10·7 ± 4·7 (3)†

The values are means ± S.E.M. of n (in parentheses) cells. † $P > 0·1$; * $P < 0·05$; ** $P < 0·001$.

after removal of the antagonist (Fig. 4*A*, 3 and 4, and Fig. 4*B*, 2 and 3), although repetitive activation of these receptors by successive applications of 100 μM kainate accelerated the rate of recovery (Fig. 4*A* and *B*). It is possible that the antagonist was trapped in the channels gated by these receptors (Fig. 4*B*, 3) and that the rate of recovery from antagonism was governed mainly by kainate-induced opening of the channels (compare Fig. 4*A* and *B*). The recovery data are consistent with the use dependence of drug dissociation from the receptor–channel complex.

The antagonism by IEM-1460 of inward currents elicited by kainate in oocytes expressing homomeric GluR1 or GluR3 receptors was voltage dependent (Table 1). Antagonism was weak at $V_h = -40$ mV, but it was made more profound by hyperpolarization. This is the direction of voltage dependence that would be expected for a positively charged channel blocker applied extracellularly.

The effects of IEM-1754. IEM-1754 also inhibited currents elicited by kainate in a voltage-dependent (Table 1) and use-dependent manner. With respect to antagonism of homomeric GluR1 receptors and GluR3 receptors, the IC_{50} was 6·0 μM at −80 mV, i.e. IEM-1754 was less potent than IEM-1460. The potency of IEM-1754 was also lower when the glutamate receptors contained the edited GluR2 subunit (data not shown).

Antagonism by adamantane derivatives of native AMPA/kainate receptors of cells freshly isolated from rat brain slices

Antagonism by IEM-1460. Inward currents of 0·13–5·4 nA induced by 100 μM kainate at −80 mV were recorded in all cells isolated from hippocampus. The brief spontaneous inward currents observed in the majority of cells (133 out of 143) supported the notion that the isolated cells were neurones rather than glia. IEM-1460 (up to 1 mM) alone had no effect on the hippocampal cells, but caused a concentration-dependent inhibition of kainate-induced inward currents ($n = 134$). Concentration–inhibition curves for IEM-1460 action on the hippocampal cells are shown in Fig. 2. Of 134 hippocampal cells tested, nineteen exhibited high sensitivity ($IC_{50} = 1·6$ μM at V_h −80 mV), forty-two intermediate sensitivity ($IC_{50} = 325$ μM at V_h −80 mV) and seventy-three low sensitivity ($IC_{50} = 1617$ μM at $V_h = -80$ mV) to antagonism by IEM-1460. In the former case the value of s was ~0·6, whereas for the intermediate- and low-sensitivity cells it was ~1·0. This low value of s for highly sensitive neurones coupled with the deviation of several data points from the theoretical curve at high IEM-1460 concentrations indicates a possible heterogeneity of receptors. If concentration–inhibition curve analysis is carried out by removing the assumption of 100% maximum inhibition, then a maximum inhibition of 85% is estimated, the value of s becomes 0·89 and the IC_{50} is decreased to 0·85 ± 0·19 μM. However, the dominant receptor population in these neurones is approximately 1000 and 200 times more sensitive to IEM-1460 than those in low- and intermediate-sensitivity groups, respectively. Figure 5 illustrates data from three cells in which agonist and antagonist were co-applied for periods of 1 s separated by intervals of 20 s. In highly sensitive cells, maximal inhibition was sometimes almost achieved during the first co-application (Fig. 5*C*), but in the majority it was obtained only after the fourth application (Fig. 5*B*). For all three groups of cells, recovery of responses to kainate after antagonism by IEM-1460 was enhanced by repeated applications of the agonist (Fig. 6*C*).

Use and voltage dependence of antagonism. Figures 5 and 6*A* and *C* demonstrate use-dependent inhibition by 20 μM IEM-1460 of kainate-induced currents elicited in hippocampal cells. For all three groups of cells the level of antagonism usually increased during repeated co-applications of agonist and antagonist (Figs 5 and 6*A*). Also, the recovery of responses to kainate was enhanced by application of the agonist, several applications of kainate

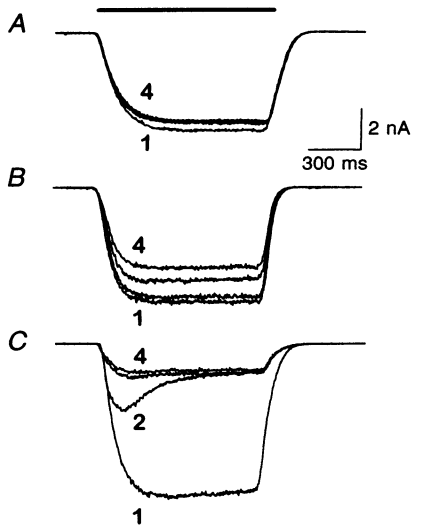


Figure 5. Identification of classes of cells isolated from rat hippocampus
Families of whole-cell currents induced by kainate (100 μM) alone (trace 1 in each family) or in combination with 20 μM IEM-1460 (traces 2–4 in each family) in three cells freshly isolated from hippocampus. Inward currents were elicited in each case by 1 s applications of kainate every 20 s at $V_h = -120$ mV. *A*, a cell with a low sensitivity to antagonist (maximal inhibition, 9%) had a pyramidal-shaped soma and dendrites. *B*, a cell of intermediate sensitivity (maximal inhibition, 28%) had a triangular-shaped soma, but lacked dendrites. *C*, a cell of high sensitivity (maximal inhibition, 80%) was oval in shape without processes. For all three cells, the 4th application of antagonist caused maximal inhibition of the kainate-induced inward current.

being required for complete restoration of control response amplitudes (Fig. 6*C*). Antagonism of the responses to kainate was also dependent on voltage, but there were qualitative differences between the groups of cells. In low-sensitivity cells antagonism did not significantly differ at −40 and −80 mV, but it increased when V_h was changed to −120 mV. In the intermediate group of cells a progressive increase in antagonism was observed over the voltage range −40 to −120 mV, whereas in the highly sensitive neurones the antagonism was maximal at −80 mV.

The effects of IEM-1754. The effects of IEM-1754 (3–100 μM) were studied in forty-one cells isolated from hippocampus. It inhibited currents elicited by 100 μM kainate in a voltage-dependent (Table 1) and use-dependent

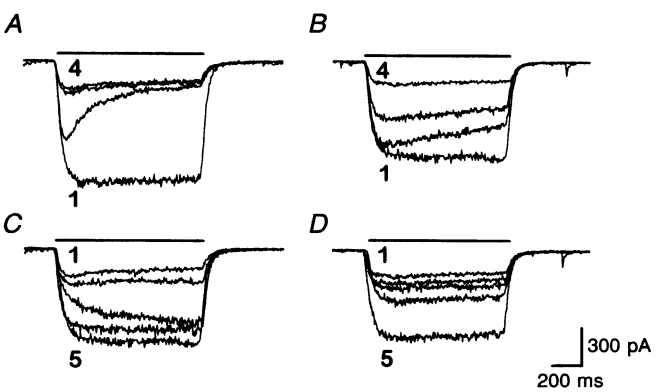


Figure 6. Use-dependent antagonism of native glutamate receptors
Comparison of use-dependent block induced by IEM-1460 and PhTX-343. The traces are families of whole-cell currents induced by kainate (100 μM), either alone or in combination with antagonist in cells freshly isolated from hippocampus. The inward currents were activated by 1 s applications of kainate every 20 s at $V_h = -120$ mV. *A*, inhibition of responses to 100 μM kainate by 20 μM IEM-1460. Trace 1 is a current elicited by kainate alone. Traces 2–4 are currents evoked during co-application of kainate and IEM-1460; they represent responses to the 1st, 3rd and 5th applications of this mixture. *B*, antagonism of responses to 100 μM kainate by 5 μM PhTX-343. Trace 1 is an inward current elicited by kainate alone. Traces 2–4 are currents evoked subsequently during co-application of kainate and PhTX-343; they represent responses to the 1st, 3rd and 16th applications of this mixture. *C*, recovery of kainate-induced currents after antagonism by 20 μM IEM-1460. Trace 1 illustrates the response elicited by co-application of kainate and antagonist. Traces 2–5 are currents evoked by subsequent applications of kainate alone; the responses to the 1st (i.e. 20 s after removal of IEM-1460), 3rd and 7th applications of kainate are illustrated. *D*, recovery of kainate-induced currents after antagonism by 5 μM PhTX-343. Trace 1 illustrates the response to co-application of kainate and 5 μM PhTX-343. Traces 2–4 represent currents evoked subsequently by kainate alone; the responses to the 1st (i.e. 20 s after removal of PhTX-343), 8th and 11th applications of kainate are illustrated. Several pulses of kainate applied at $V_h = +30$ mV increased the rate of recovery from antagonism by PhTX-343; responses to kainate were recorded at $V_h = -120$ mV (trace 5). The data in *A*–*D* were obtained from the same cell.

manner (not shown). Twenty-five of the cells exhibited low sensitivity, nine intermediate sensitivity and seven high sensitivity to the antagonist. IEM-1754 and IEM-1460 were equipotent when applied to low- and intermediate-sensitivity cells, but when tested on high-sensitivity cells at −80 mV, IEM-1754 was a significantly weaker antagonist than IEM-1460. The voltage dependence of antagonism by IEM-1754 was similar to that of IEM-1460 for cells of low and intermediate sensitivity (Table 1), but there were marked differences between the two drugs with respect to the high-sensitivity neurones. For example, antagonism by IEM-1754 was less sensitive to changes of V_h between −40 and −80 mV, but it was markedly reduced by hyperpolarization to −120 mV ($P < 0.003$). These differences require further investigation.

Comparison with the blocking action of PhTX-343. The antagonism of kainate-induced currents by PhTX-343 (0·1–25 μM) was studied in eight hippocampal cells. PhTX-343 induced a concentration-dependent inhibition of these currents in all eight cells. In three cells, 5 μM PhTX-343 inhibited the response to 100 μM kainate by 17·3 ± 6·8%, whereas in three other cells the inhibition was 78·3 ± 11·0% (Fig. 6*B*). The two remaining cells manifested an intermediate sensitivity, i.e. responses to 100 μM kainate were inhibited by ~46%. Figure 6 illustrates the relatively slow kinetics of PhTX-343 inhibition compared with those of IEM-1460 and IEM-1754. The first co-application of 100 μM kainate and 20 μM IEM-1460 induced 75% inhibition of the kainate-induced inward current, which increased only slightly during subsequent co-applications of antagonist and agonist. In contrast, the first co-application of 100 μM kainate and 5 μM PhTX-343 caused only a 32% inhibition, which increased with subsequent co-applications to reach a maximum (75%) after eleven co-applications. The inhibitory effect of PhTX-343 could not be eliminated completely even after extensive washes with saline containing 100 μM kainate. Comparison of traces 1 and 4 in Fig. 6*D* indicates that recovery from inhibition by PhTX-343 did not exceed 50% after the 11th application of kainate alone. The rate of recovery from inhibition was enhanced after agonist was added at $V_h = +30$ mV (Fig. 6*D*, trace 5).

DISCUSSION

The two adamantane derivatives are potent antagonists of a number of types of receptor that gate cationic channels, including nicotinic acetylcholine receptors of frog neuromuscular junction, ionotropic glutamate receptors of insect muscle and NMDA receptors of cultured cortical neurones (Antonov *et al.* 1995). The data presented herein show that these compounds are also potent non-competitive antagonists of native AMPA/kainate receptors and recombinant AMPA receptors, but that their potency as antagonists of these receptors is greatly reduced by the presence of one or more edited GluR2 subunits. An arginine residue at the Q/R site in the second membrane segment of GluR2 has been shown previously to influence the potencies of non-competitive antagonists of mammalian, recombinant AMPA receptors (Blaschke *et al.* 1993; Brackley *et al.* 1993; Herlitze *et al.* 1993). Extensive studies of recombinant receptors of this type have revealed the importance of the edited GluR2 subunit in determining the properties of the channels that they gate (see reviews by Wisden & Seeburg, 1993; Jonas & Burnashev, 1995). For example, the presence of edited GluR2 subunits prevents the permeation of Ca^{2+} (Hume *et al.* 1991; Burnashev *et al.* 1992). The inhibitory actions of the adamantane derivatives on kainate responses evoked in oocytes injected with mRNAs for one or more of GluR1–3 subunits and in cells isolated from rat CNS were similar, and indicative of open channel block. Inhibition developed only in the presence of agonist (kainate), i.e. activation of the receptor was an indispensable condition for antagonism, and it was use dependent, presumably because equilibrium between blocked and unblocked channels was attained slowly. The latter could be most clearly seen during co-application of kainate and antagonist in experiments on homo-oligomeric GluR1 receptors (Fig. 4) and on hippocampal cells (Fig. 5). One explanation is that the antagonist molecule becomes trapped in the channel (Neely & Lingle, 1986) gated by the recombinant GluR1 receptor and native AMPA/kainate receptors lacking edited GluR2 subunits. This relatively stable blocked state could be reversed (albeit not completely in the case of PhTX-343) by applying kainate (Figs 4 and 6). Complete recovery from PhTX-343 blockade was readily achievable if agonist was applied during step depolarizations (Fig. 6*D*). Antagonism was also voltage dependent, i.e. it became more profound with hyperpolarization. This would be expected for open channel block by a positively charged molecule applied extracellularly. Indeed IEM-1460 and IEM-1754 would be predominantly doubly charged at physiological pH. The quarternized amine group on IEM-1460 is permanently charged and this may be the reason for its higher potency compared with IEM-1754. Kinetics of the blocking action and its voltage dependence in the different groups of neurones are under study.

The list of drugs that block the open channels gated by AMPA/kainate receptors is very short. A voltage-dependent block of non-NMDA receptors expressed in *Xenopus* oocytes injected with rat brain RNA by millimolar concentrations of tetrabutylammonium was observed by Randle (1990), and micromolar concentrations of polyamine amides, such as PhTX-343 (Brackley *et al.* 1993), ArgTX-636 (Brackley *et al.* 1993; Herlitze *et al.* 1993) and JSTX (Tsubokawa *et al.* 1995; Iino *et al.* 1996), have been shown to block channels of native AMPA/kainate receptors and recombinant AMPA receptors of this type that lack edited GluR2 subunits. To this short list may now be added the two adamantane derivatives that are the subject of this report. These compounds are potentially valuable tools for identifying classes of AMPA/kainate receptors and their roles in synaptic transmission. The rapid onset of and recovery from antagonism elicited by the two adamantane

derivatives compared with ArgTX-636 and PhTX-343 may be advantageous in these respects. IEM-1460 seems to be a preferential tool: it is several times more potent than IEM-1754 inhibiting recombinant AMPA receptors and native AMPA/kainate receptors lacking edited GluR2, whereas it is 5·6 times less potent inhibiting NMDA receptors (Antonov *et al.* 1995).

It is known that the low Ca^{2+} permeability of channels gated by some AMPA/kainate receptors is determined by the amino acid residue at the Q/R site in the M2 membrane segment of these receptors (Burnashev *et al.* 1992; Dingledine, Hume & Heinemann, 1992; Kohler, Burnashev, Sakmann & Seeburg, 1993), and there is some evidence to support the idea proposed by Brackley *et al.* (1993) that this site hinders access of open channel blockers to their binding site(s) in the channel. The suspected presence of specific AMPA/kainate receptor subunits has been revealed in a variety of neurones using *in situ* hybridization (Hollmann & Heinemann, 1994) and immunocytochemistry (Petralia & Wenthold, 1992). The edited GluR2 subunit is abundant in many principal neurones of adult rat brain, such as pyramidal cells in hippocampus or neocortex, granule cells in gyrus dentatus and hilar mossy cells, but it is absent or present at only low levels in interneurones of these brain regions. The relative abundance of the edited GluR2 subunit is inversely correlated with high Ca^{2+} permeability of AMPA/kainate receptor channels (Bochet *et al.* 1994; Jonas *et al.* 1994; Geiger *et al.* 1995). In this study, many of the cells isolated from the rat hippocampus that exhibited low sensitivity to antagonism by the adamantane derivatives had morphological characteristics of pyramidal neurones (i.e. pyramidal-shaped somata and apical dendrites). It is assumed that the AMPA/kainate receptors expressed by these cells contain edited GluR2 subunits, although there is evidence that some pyramidal neurones express a minor population of receptors lacking the edited GluR2 subunit (Wenthold, Petralia, Blahos & Niedzielski, 1996). In contrast, those cells that were most sensitive to antagonism were round or oval in shape and lacked other morphological characteristics of pyramidal neurones. An immunocytochemical study by Buldakova & Samoilova (1997) suggests that these are GABAergic interneurones. The cells exhibiting intermediate sensitivity to the antagonists were probably also interneurones, but belonging to a separate population. It has been shown recently that interneurones are heterogeneous in terms of their Ca^{2+} permeabilities (McBain & Dingledine, 1993; Isa, Itazawa, Iino, Tsuzuki & Ozawa, 1996) and edited GluR2 subunit expression (Leranth, Szeidemann, Hsu & Buzsaki, 1996). We did not observed any differences in the current–voltage relationships of the three groups of isolated cells, although Isa *et al.* (1996) have classified hippocampal neurones into three groups on the basis of their rectification properties during kainate application. This difference may be due to the dialysis of spermine from neurones under whole-cell recording conditions (Isa, Iino, Itazawa & Ozawa, 1995; Koh, Burnashev & Jonas, 1995).

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