0026-895X/96/030540-07$3.00/0

MOLECULAR PHARMACOLOGY, 49:540–546 (1996).

Antagonist Pharmacology of Kainate- and α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid-Preferring Receptors

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Received October 12, 1995; Accepted November 28, 1995

SUMMARY

Whole-cell recordings were used to study the antagonist pharmacology of two subtypes of non-*N*-methyl-D-aspartate glutamate receptors: the kainate-preferring subtype expressed by rat dorsal root ganglion (DRG) neurons and the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-preferring subtype expressed by neurons from rat cerebral cortex. A series of quinoxaline derivatives were tested for the ability to distinguish between AMPA and kainate receptors, as determined by differential potency. Of the nine compounds studied, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (NBQX) showed the highest selectivity for AMPA-preferring receptors, whereas 5-chloro-7-trifluoromethyl-2,3-quinoxalinedione (ACEA-1011) showed the highest selectivity for the kainate-preferring subtype. NBQX blocked non-*N*-methyl-D-aspartate currents in cortical cells with a K_b of 0.3 μM, but in DRG neurons the K_b for NBQX was 3-fold higher (0.9 μM). ACEA-1011 also blocked the currents in DRG cells with a K_b of ~1 μM, but in cortical neurons the K_b for this drug was 10–12 μM. Several additional compounds were tested for selective potency, including 5-nitro-6,7,8,9-tetrahydrobenzo[*G*]indole-2,3-dione-3-oxime, γ-D-glutamylaminomethylsulphonic acid, and derivatives of kynurenic acid and 1-benzazepine. 5-Nitro-6,7,8,9-tetrahydrobenzo[*G*]indole-2,3-dione-3-oxime displayed the highest selectivity in this group, blocking kainate receptors with a K_b of 6 μM while inhibiting AMPA receptors with a K_b of >100 μM. The remaining antagonists showed <3-fold selectivity between AMPA and kainate receptor subtypes. Our results suggest that most competitive antagonists block native AMPA and kainate receptors with approximately similar potencies, which is in marked contrast to the substantial differences in potency that have been observed with receptor agonists.

The glutamate-gated ion channels make up three different subfamilies named for the agonists NMDA, AMPA, and kainate. These subtypes were originally defined by pharmacological studies (1), but recent work on cloned GluR subunits has confirmed that NMDA, AMPA, and kainate receptors are distinct molecular entities (2, 3). Comparison of physiological responses evoked at native receptors with those obtained with cloned receptor subunits expressed in heterologous cells indicates that AMPA receptors are formed by the subunits GluR1–4 (4, 5), whereas kainate receptors are constructed from GluR5–7 (6–9) in combination with the KA1 and KA2 subunits (10–12). Current understanding of the functional role of these different receptor subtypes is based largely on studies involving selective agonist and antagonist compounds. Both NMDA and AMPA receptors have been shown to mediate rapid excitatory transmission in the CNS, but the role of kainate receptors in neuronal function remains poorly understood.

Early work with kainate demonstrated that cells in hippocampus (13) and the axons of primary sensory neurons (14) are highly sensitive to this compound. Subsequent studies have focused on these cell types to characterize the properties of native kainate receptors. Sensory neurons do not express functional NMDA or AMPA receptors, but a subset of small-diameter DRG neurons produce kainate receptors (15, 16). Molecular studies indicate that the GluR5 and KA2 subunits are expressed at high levels in DRGs (2, 6, 12), and the currents recorded in heterologous cells supplied with these subunits display many of the same physiological properties as native kainate receptor channels in freshly isolated DRG

This work was supported by National Institutes of Health Grant NS30888 and by the McDonnell Center for Cellular and Molecular Neurobiology.

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; DRG, dorsal root ganglion; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluR, glutamate receptor; ACEA-1011, 5-chloro-7-trifluoromethyl-2,3-quinoxalinedione; ACEA-1021, 5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione; 8ClDDHB, 8-chloro-2,5-dihydro-2,5-dioxo-3-hydroxy-1*H*-benzazepine; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; 5,7diClKyn, 5,7-dichlorokynurenic acid; 6,7diClQX, 6,7-dichloroquinoxaline-2,3-dione; GAMS, γ-D-glutamylaminomethylsulphonic acid; HQXCA, 3-hydroxyquinoxaline-2-carboxylic acid; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline; NS-102, 5-nitro-6,7,8,9-tetrahydrobenzo[g]indole-2,3-dione-3-oxime; 5NQX, 5-nitroquinoxaline-2,3-dione; QX, quinoxaline-2,3-dione; $I_{control}$, current evoked by 10 mM kainate in the absence of antagonist; CNS, central nervous system; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

neurons (2, 8, 12). Subunit localization studies in the brain suggest that hippocampal neurons express a number of different kainate receptor mRNAs (6, 7, 9, 10, 12); however, physiological detection of kainate receptors in hippocampal neurons and other CNS cell types has often proved difficult (16, 17). This difficulty arises because CNS neurons both *in vivo* (17) and in culture (18, 19, 20) seem to express a much higher density of AMPA receptors than kainate receptors. In addition, many kainate receptor agonists, including kainate and domoate, also serve as relatively potent agonists for AMPA receptors (18). Further progress toward understanding the function of neuronal kainate receptors clearly would be aided by the development of better pharmacological reagents. Previous work has identified a number of compounds that display some degree of selectivity between AMPA and kainate receptors, including the noncompetitive 2,3-benzodiazepines GYKI 52466 and 53655 (20, 21), as well as competitive drugs such as the oxime NS-102 (22). However, many non-NMDA antagonists have not been directly evaluated for subtype selectivity in physiological assays on native receptors.

In the current study, we determined the relative potency of 13 competitive antagonists at AMPA receptors expressed by cultured rat cortical neurons and at kainate receptors in freshly dissociated rat DRG neurons. Our results show that most competitive antagonists display relatively weak selectivity between AMPA and kainate receptors and are therefore likely to block both subtypes when applied to neurons *in situ*.

Materials and Methods

Cell preparation

DRG cells were dissociated as described by Wilding and Huettner (21). The cells were maintained overnight at room temperature in Earl's balanced salt solution (14160, GIBCO-BRL; containing 2 mM $CaCl_2$, 1 mM $MgSO_4$, 20 mM glucose, and 26 mM $NaHCO_3$, equilibrated with 5% CO_2/95% O_2). For most experiments, recordings were obtained the day after dissociation. Cortical cells were isolated from newborn Long-Evans rats and maintained in culture as described previously (23). Recordings were obtained from cortical neurons after 6–14 days in culture.

Electrical recording and drug application. Whole-cell recordings of agonist-gated currents were obtained with an Axopatch 200 amplifier (Axon Instruments). Pipette resistance ranged from 1 to 10 MΩ with an internal solution containing 10 mM HEPES, 10 mM EGTA, 5 mM CsCl, and 140 mM $CsCH_3SO_3$ or CsF, titrated to pH 7.40 with CsOH. In most cases, current recorded during agonist applications was compressed by averaging 3 msec of data at 0.1–1-sec intervals. The recording chamber was continuously perfused with Tyrode's solution (150 mM NaCl, 4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, 10 mM HEPES, pH 7.4). The external solution for drug application contained 160 mM NaCl, 2 mM $CaCl_2$, 10 mM HEPES, pH 7.4, 500 nM tetrodotoxin, and 2 μM MK-801. Control, agonist, and antagonist solutions were applied from a bank of microcapillary tubes mounted on a micromanipulator and connected to a series of reservoirs. Solution flow was driven by gravity. To block desensitization of kainate currents in DRG cells (15), concanavalin A was applied at 2 μM for 5–10 min before recording. Concentration-response curves were generated and analyzed as described previously (24). The dissociation constant for each inhibitor was calculated from the displacement it produced in the EC_{50} for receptor activation by kainate. More extensive Schild analysis was not undertaken due to the low solubility of many of the antagonists and their apparent low selectivity based on analysis of single doses.

Antagonists were prepared as 10–40 mM stock solutions in DMSO, ethanol (dizocilpine), or 50% DMSO/50% ethanol (NS-102). These stocks were diluted into control or agonist-containing solutions so that the final concentration of vehicle was ≤0.5%. Pure DMSO, ethanol, or DMSO/ethanol was added to solutions lacking antagonist so that all of the solutions used in an experiment would contain the same levels of vehicle. All drug stock solutions were stored at −20°. AMPA, GAMS, and CNQX were purchased from Research Biochemicals International. Kainate, L-glutamic acid, tetrodotoxin, and concanavalin A were obtained from Sigma Chemical Co. QX and HQXCA were purchased from Aldrich. 6ClQX, 6,7diClQX, and 5NQX were obtained from Tocris Cookson. The remaining drugs were kindly provided by the following companies: ACEA Pharmaceuticals, a wholly owned subsidiary of CoCensys (ACEA-1011, ACEA-1021, and 8ClDDHB), Marion Merrell Dow (5,7diClKyn), Merck, Sharp, and Dohme (dizocilpine), Novo Nordisk (NBQX), and NeuroSearch (NS-102).

Results

Quinoxaline derivatives. Whole-cell currents activated by kainate were used to study the inhibitory potency of a series of compounds at kainate- and AMPA-preferring receptors. Current through AMPA-preferring receptors was recorded in cultured neocortical neurons, whereas kainate receptors were studied in freshly dissociated DRG neurons. Previous studies (e.g., Ref. 25) have shown that kainate activates large maintained currents through AMPA receptors in CNS neurons. For most experiments, DRG neurons were briefly exposed to concanavalin A at the beginning of the recording session. Treatment with concanavalin A has been shown to eliminate desensitization of kainate receptors without causing any significant change in the agonist concentration required to give half-maximal receptor activation (15, 18). By treating the cells with concanavalin A, we were able to study the steady state inhibition produced by the various antagonists, which would otherwise have been difficult to resolve once receptors were strongly desensitized. The inhibitory action of each antagonist was verified qualitatively in freshly isolated cells that had not been exposed to lectin. We saw no evidence that exposure to concanavalin A had any effect on the relative potency of the antagonists.

Initially, four quinoxalinediones, CNQX, NBQX, ACEA-1011, and ACEA-1021, were tested for their ability to inhibit kainate currents in DRG and cortical neurons. To determine antagonist potency and provide information on the mechanism of inhibition, we examined the effect of a fixed concentration of each antagonist on the concentration-response relation for kainate. Fig. 1 shows the inhibition of whole-cell kainate current by NBQX (26) and by ACEA-1011 (27, 28). In the absence of antagonist, kainate currents in cortical neurons were half-maximal at a concentration of 160 μM, whereas in DRG cells, the half-maximal concentration was 6 μM. In both cell types, the dose-response relation for kainate was shifted toward higher concentrations by the inclusion of NBQX or ACEA-1011. Inhibition by the drugs was overcome with saturating doses of kainate, which is consistent with a competitive mechanism of antagonism.

Comparison among the first four compounds that we tested revealed that all four drugs blocked with similar potency at kainate receptors ($K_b \cong 1$ μM), but they displayed a much broader range of potencies against AMPA receptors ($0.3 \leq K_b$

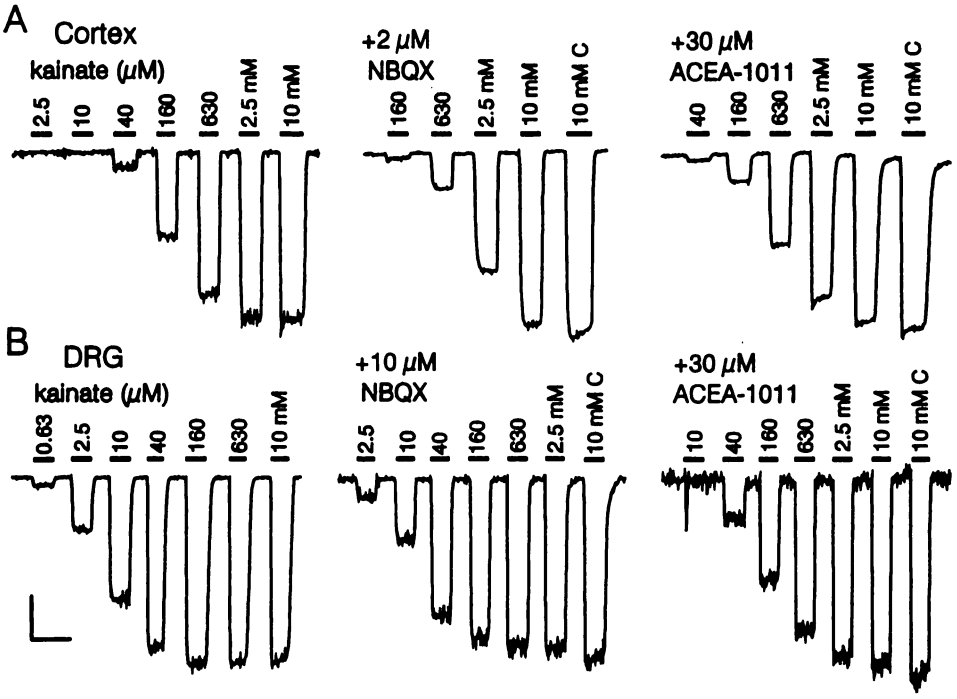

Fig. 1. Antagonism of whole-cell kainate currents by NBQX and ACEA-1011. A, Currents activated by kainate alone (*left*), kainate plus 2 μM NBQX (*middle*), and kainate plus 30 μM ACEA-1011 (*right*) in three different cortical neurons. *Filled bars*, duration of agonist exposure. *Numbers above each bar*, kainate concentration (in μM). A control response to 10 mM kainate alone (*10 mM C*) is shown at the end of each antagonist series. B, Currents evoked by kainate alone (*left*), kainate plus 10 μM NBQX (*middle*), and kainate plus 30 μM ACEA-1011 (*right*) in three different DRG neurons that had been treated with concanavalin A to block desensitization. Holding potential, −70 mV. *Scales left to right*: (A) 100 pA, 35 sec; 150 pA, 20 sec; 250 pA, 20 sec; and (B) 200 pA, 25 sec; 50 pA, 40 sec; 50 pA, 25 sec.

≥ 12) (Table 1 and Fig. 2). In terms of subtype selectivity, NBQX was ~3-fold more potent against AMPA-preferring receptors, CNQX showed nearly identical potency at both receptors, and ACEA-1021 and ACEA-1011 were ~3-fold and 10–12-fold more potent at kainate-preferring receptors, respectively. Based on these observations, we tested a number of additional quinoxaline derivatives in the hope of finding a compound with micromolar potency against kainate receptors but much lower affinity for the AMPA subtype. As shown in Fig. 2 and summarized in Table 1, all five of these additional compounds (QX, 6ClQX, 6,7diClQX, 5NQX, and HQXCA) were somewhat selective for kainate versus AMPA receptors, but none of the drugs showed >5-fold difference in potency at the two receptor subtypes (see Fig. 5).

Other antagonists. In addition to the quinoxaline derivatives, we tested several other compounds that have been shown to exhibit some degree of differential potency between AMPA and kainate. The compound GAMS was a relatively weak antagonist of kainate current in both cell types (Fig. 3 and Table 1). The K_b for GAMS was 360 μM in DRG neurons and 750 μM in cortical cells, yielding a selectivity ratio of 2.1. Because this degree of selectivity was considerably lower than that obtained in several previous studies (29–31), we also tested cortical neurons for the action of GAMS against steady state currents gated by AMPA (Fig. 4D). In all six of the cells tested, GAMS inhibited the current evoked by low doses of AMPA. The K_b value for inhibition of steady state AMPA current was ~400 μM. In two of the cells (e.g., Fig.

TABLE 1

Activation of AMPA and kainate receptors in the presence and absence of antagonists

EC_{50} values and slope factors (n) were obtained from the best fit of $I/I_{control} = 1/(1 + (EC_{50}/[kainate])^n)$ to concentration response data, where $I_{control}$ was the current evoked by 10 mM kainate without antagonist. K_b values were calculated from the relationship $K_b = [antagonist]/[(EC_{50}'/EC_{50}) - 1]$, where EC_{50}' is the half-maximal dose of agonist in the presence of a fixed antagonist concentration.

Antagonist	Cortex					DRG					Selectivity ratio: cortex K_b/ DRG K_b
	Dose	Kainate EC_{50}	Slope factor	Antagonist K_b	No. of cells	Dose	Kainate EC_{50}	Slope factor	Antagonist K_b	No. of cells	
	μM	μM		μM		μM	μM		μM		
None		160	1.3		10		6.0	1.0		15	
Quinoxaline derivatives											
CNQX	10	1400	1.5	1.3	8	10	45	0.9	1.5	6	0.9
NBQX	2	1300	1.7	0.3	4	10	70	0.9	0.9	3	0.3
ACEA-1011	30	550	1.2	12	8	30	190	0.9	1.0	13	12
ACEA-1021	5	474	1.3	2.5	6	10	73	0.8	0.9	8	2.8
QX	1000	1000	1.1	190	6	1000	117	0.7	54	5	3.5
6ClQX	100	407	1.1	65	6	100	22	1.1	38	5	1.7
6,7diClQX	30	343	1.2	26	6	30	20	1.2	13	6	2.0
5NQX	500	442	1.1	284	7	500	34	1.0	107	6	2.7
HQXCA	1000	865	1.4	227	7	1000	53	1.0	128	7	1.8
Other antagonists											
NS-102	10	174	1.2	114	7	10	16	1.0	6	6	19
5,7diClKyn	30	385	1.2	21	8	30	15	1.0	20	6	1.1
8ClDDHB	50	931	1.2	10	8	50	35	0.9	10	6	1.0
GAMS	5000	1230	1.6	748	6	5000	90	1.0	357	6	2.1

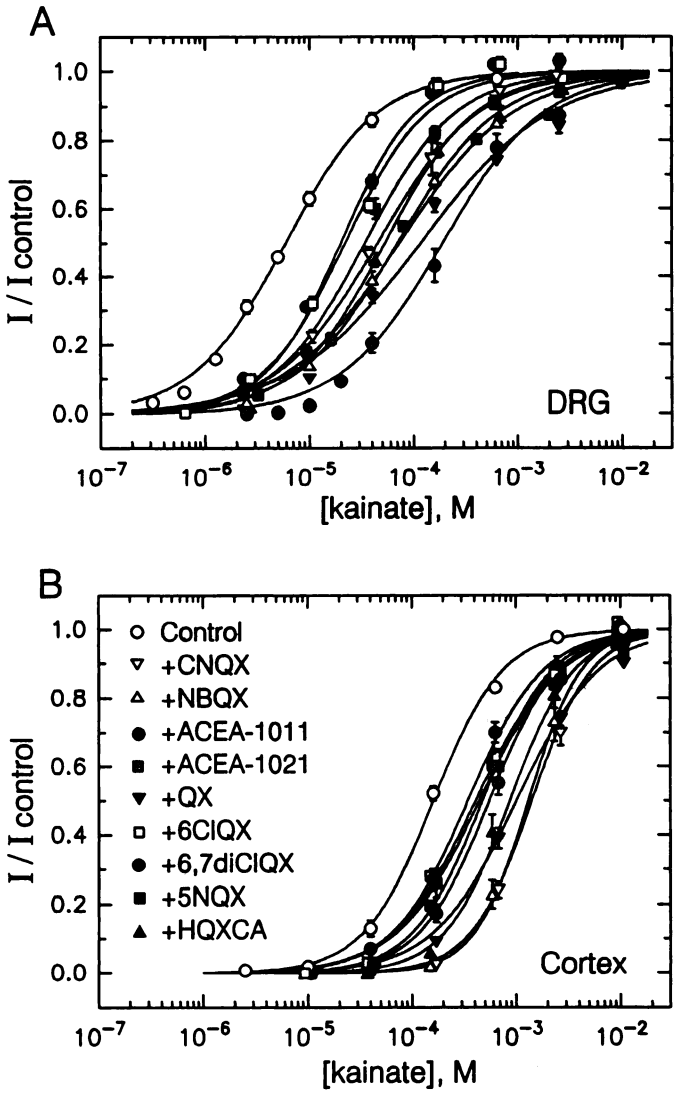

Fig. 2. Effect of quinoxaline derivatives on the concentration-response relation for kainate in DRG (A) and cortical (B) neurons. Data are mean ± standard error of the normalized currents ($I/I_{control}$). *Smooth curves*, best fit of $I/I_{control} = 1/(1+(EC_{50}/[kainate])^n)$ to all of the data points for each concentration-response relation. Curve parameters and antagonist concentrations are given in Table 1 (*n* is the slope factor). Data for cortical cells tested with ACEA-1021 come from Ref. 24, and data for five of the eight cortical cells tested with ACEA-1011 come from Ref. 28.

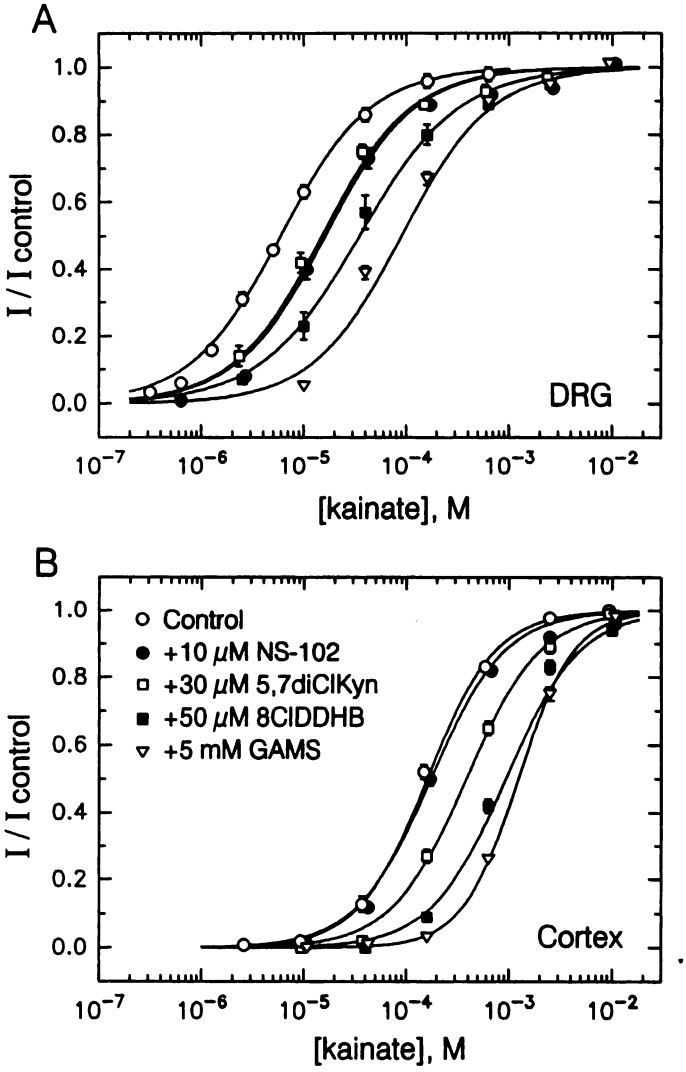

Fig. 3. Effect of various antagonists on the concentration-response relation for kainate in DRG (A) and cortical (B) neurons. Data are mean ± standard error of the normalized currents ($I/I_{control}$). *Smooth curves*, best fit of $I/I_{control} = 1/(1+(EC_{50}/[kainate])^n)$ to all of the data points for each concentration-response relation. Curve parameters and antagonist concentrations are given in Table 1 (*n* is the slope factor).

4D), the response to saturating AMPA (250 μM) was slightly potentiated in the presence of GAMS (1 mM), a phenomenon that has been previously described (32, 33). Taken together, these results indicate very little selectivity by GAMS between AMPA and kainate subtypes.

Significantly greater selectivity was observed for NS-102 (22). As shown in Fig. 3, 10 μM NS-102, which is close to the limit of aqueous solubility, produced a 3-fold shift in the EC_{50} for kainate in DRG neurons, suggesting a K_b of 5–6 μM for this drug. In cultured cortical neurons, 10 μM NS-102 consistently produced a slight reduction in current gated by 40 μM to 2.5 mM kainate, but the data in Fig. 3 indicate a K_b of ≥100 μM at cortical receptors (see Discussion). These results suggest an 18–20-fold selectivity in favor of the kainate-preferring receptors (Table 1). Because the concentration-response relations for kainate alone and kainate plus NS-102 were collected in different cells, we were concerned that the small shift observed with NS-102 in cortical cells might simply reflect random variability in the responses to kainate and not a direct inhibitory action by the drug. To control for this possibility, several neurons were tested with various concentrations of kainate alone, as well as kainate plus NS-102 (Fig. 4, A and B). In six cortical cells, 10 μM NS-102 produced 6 ± 2% inhibition of currents gated by 160 μM kainate, which is consistent with our estimated dissociation constant of 114 μM. Similar experiments performed with AMPA instead of kainate gave the same result (Fig. 4C); 10 μM NS-102 produced ~6–10% inhibition against concentrations of AMPA near the EC_{50} (5 μM) but did not inhibit the steady state current evoked by a saturating dose of AMPA (250 μM).

Finally, both the 5,7-dichloro derivative of kynurenic acid and the 8-chloro derivative of dihydro-2,5-dioxo-3-hydroxy-1*H*-benzazepine produced modest inhibition of kainate current in DRG and cortical neurons (34, 35). Neither drug, however, displayed significant selectivity between the two receptor subtypes (Fig. 3 and Table 1).

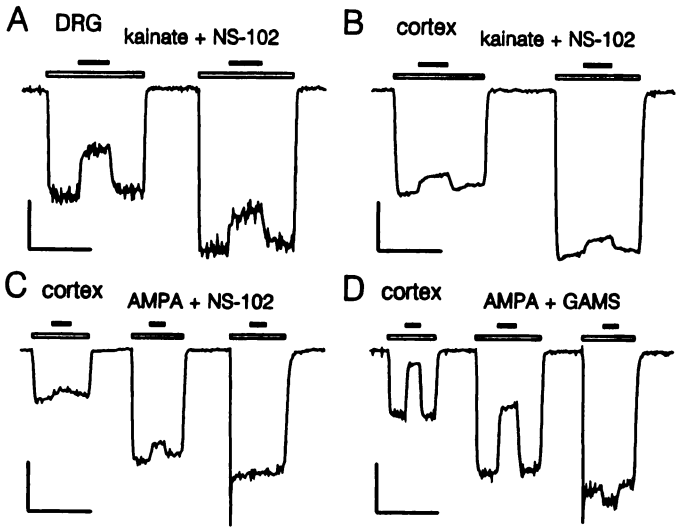

Fig. 4. Inhibition of whole-cell currents by NS-102 and GAMS. A, *Open bars*, application of 10 μM (*left*) and 40 μM (*right*) kainate to a DRG neuron; *closed bars*, coapplication of 10 μM NS-102. B, *Open bars*, application of 160 μM (*left*) and 630 μM (*right*) kainate to a cortical neuron; *closed bars*, coapplication of 10 μM NS-102. C and D, Currents evoked by 4, 16, and 250 μM AMPA in two different cortical neurons. *Closed bars*, coapplication of 10 μM NS-102 (C) or 1 mM GAMS (D). Note the slight potentiation of current evoked by 250 μM AMPA during the period of coapplication with GAMS. Holding potential, −60 mV. *Scale bars:* (A) 100 pA, 30 sec; (B) 300 pA, 40 sec; (C) 150 pA, 30 sec; and (D) 100 pA, 30 sec.

Discussion

Competitive antagonists. Recent physiological studies of AMPA- and kainate-preferring receptor pharmacology have highlighted their differential sensitivity to modulation by cyclothiazide (2, 16) and to inhibition by noncompetitive 2,3-benzodiazepines (20, 21). We undertook a broad comparison of competitive antagonists against AMPA and kainate receptors in the hope of identifying drugs capable of selectively inhibiting one subtype or the other. AMPA receptors were studied in cultured rat cortical neurons, whereas freshly isolated DRG neurons were used to examine the pharmacology of kainate receptors. In contrast to non-NMDA receptor agonists, which exhibit ≤140-fold differences in potency at native AMPA and kainate receptors (18), we found that most competitive antagonists display only modest selectivity.

All 13 of the compounds examined in this study produced inhibition of kainate currents recorded in both DRG neurons and cultured cortical cells. Consistent with a competitive mechanism of antagonism, each drug produced a rightward shift in the concentration-response relation for kainate. In DRG cells, the slopes of the dose-response relations ranged from 0.7 to 1.2, whereas in cortical cells the range of slope factors was from 1.1 to 1.7 (15, 18). For both cortical neurons and DRG cells, there was a progression in apparent affinity from NBQX, which blocked with highest potency, to GAMS, which was clearly the weakest antagonist (36–38). The following potency sequence was obtained in cortical neurons: NBQX (1) > CNQX (4.3) > ACEA 1021 (8.3) > 8ClDDHB (33) > ACEA 1011 (40) > 5,7diClKyn (70) > 6,7diClQX (87) > 6ClQX (217) > NS-102 (380) > QX (633) > HQXCA (757) > 5NQX (947) > GAMS (2500), where the numbers in parentheses indicate the ratio of apparent dissociation constants relative to NBQX (K_b = 0.3 μM). In DRG cells, NBQX was only slightly less potent (K_b = 0.9 μM), and the full sequence of antagonist potency covered a narrower range of apparent affinities: NBQX (1) = ACEA 1021 (1) ≅ ACEA 1011 (1.1) > CNQX (1.7) > NS-102 (6.7) > 8ClDDHB (11) > 6,7diClQX (14) > 5,7diClKyn (22) > 6ClQX (42) > QX (60) > 5NQX (119) > HQXCA (142) > GAMS (397). As a result, drugs with lower affinity in cortical neurons, such as ACEA-1011 and NS-102, showed the highest selectivity for receptors expressed by DRG cells (Fig. 5). With the ratio of antagonist dissociation constants in cortical cells versus DRG neurons used as a gauge of relative potency, the following selectivity sequence is derived: NBQX (0.3) > CNQX (0.9) > 8ClDDHB (1) > 5,7diClKyn (1.1) > 6ClQX (1.7) > HQXCA (1.8) > 6,7diClQX (2.0) > GAMS (2.1) > 5NQX (2.7) > ACEA-1021 (2.8) > QX (3.5) > ACEA-1011 (12) > NS-102 (19), where the number in parenthesis is cortex K_b/DRG K_b.

NS-102 showed the highest selectivity for kainate-preferring receptors among the 13 antagonists that we tested. This compound displaces kainate from low affinity binding sites in rat cortical membranes (39) and was found to antagonize kainate-preferring receptors on embryonic hippocampal neurons (40). Selective inhibition by NS-102 also has been observed for receptors expressed in 293 cells. Verdoorn *et al.* (22) found that homomeric channels formed by the kainate receptor subunit GluR6 were significantly more sensitive to block by NS-102 than heteromeric receptors formed by coexpression of the AMPA receptor subunits GluR2 and GluR4. Because NS-102 is poorly soluble in aqueous solutions, we did not test any concentration of >10 μM. In DRG cells, this dose produced a shift of ~3-fold in the EC_{50} for kainate, whereas in cortical neurons, the effect was much smaller (see Fig. 2). The ratio of apparent dissociation constants for NS-102 favored inhibition at kainate receptors by nearly 20-fold, although in terms of absolute affinity NS-102 was 6-fold less potent at kainate receptors than were several of the quinoxalinediones (Fig. 5).

ACEA-1011 showed ~12-fold selectivity for kainate-preferring versus AMPA-preferring receptors. This compound, along with the related drug ACEA-1021, displays even higher

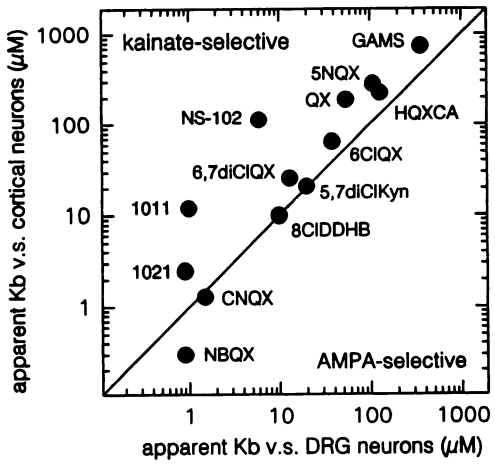

Fig. 5. Comparison of apparent K_b values against AMPA-preferring and kainate-preferring receptors. Data are K_b values determined from the shift in EC_{50} for kainate in cortical neurons (AMPA-preferring) and DRG cells (kainate-preferring). *Solid line*, equal potency against both receptors; *points above*, higher affinity for kainate receptors; *points below*, higher potency against AMPA receptors.

affinity for the glycine modulatory site on the NMDA receptor than for either of the non-NMDA receptors (24, 28). ACEA-1011 blocks glycine potentiation of NMDA receptors with a K_b of ~0.5 μM (27, 28), whereas ACEA-1021 is considerably more potent ($K_b \cong 5$ nM) (24). As expected from previous work (26, 41, 42), NBQX and CNQX were the most potent antagonists against AMPA receptors in rat cortical neurons. Our apparent dissociation constants for these drugs agree well with earlier results with neuronal receptors (41, 42), although several studies of non-NMDA receptors expressed in oocytes have reported somewhat lower K_b values (28, 31, 38, 43). In terms of subtype selectivity, NBQX was ~3-fold more potent against AMPA-preferring receptors, whereas CNQX blocked AMPA and kainate receptors with nearly identical potencies (*c.f.* 37). Indeed, the quinoxaline derivatives, as a group, showed a much tighter range of potency against kainate receptors in DRG neurons (140-fold) compared with a 950-fold range in apparent affinity for AMPA-preferring receptors in cortical cells.

A number of previous reports have suggested that the relatively weak antagonist GAMS may distinguish between AMPA and kainate receptors (29, 30). Most recently, a study of receptors expressed in *Xenopus* oocytes from whole chick brain mRNA found that GAMS blocked kainate currents with an apparent dissociation constant of ~50 μM but had virtually no effect on currents evoked by AMPA (31). Our results in cells from rat cortex and DRGs indicate substantially lower selectivity. Using kainate as the agonist, we obtained K_b values of 360 μM in DRG neurons and 750 μM in cortical cells. Further work will be needed to understand the basis for this apparent difference between native receptors expressed by a specific population of cells and those expressed in oocytes after injection of whole-brain mRNA.

Comparison with agonists. In contrast to the current study, in which we found relatively low selectivity between AMPA and kainate receptors among competitive antagonists, previous work has shown that agonist compounds can display wide differences in their relative affinity for the two receptor subtypes. Wong *et al.* (18) showed that different substitutions at the 5 position of (*S*)-willardine produced substantial changes in agonist potency. In particular, 5-substitution with hydrophobic groups greatly favored activation of kainate receptors but diminished affinity for AMPA receptors. Among the derivatives they examined, (*S*)-5-trifluoromethylwillardine showed the highest potency at kainate receptors (EC_{50} = 74 nM) and (*S*)-5-iodowillardine displayed the highest selectivity for kainate receptors relative to AMPA receptors (~140-fold) (18). Comparison across all of the agonists studied by Wong *et al.* (18) revealed that the range of EC_{50} values from the most to the least potent compound was nearly 1000-fold at kainate receptors expressed by DRG neurons versus only 170-fold at AMPA receptors in hippocampal neurons. As noted above, the opposite trend was observed for antagonist compounds in the current study: antagonist potency covered a 2500-fold range at cortical AMPA receptors but only a 400-fold range at kainate receptors. In addition, there was no obvious correlation between hydrophobic substitutions and antagonist selectivity.

Functional implications. Since their initial characterization several years ago (26, 44), quinoxalinediones such as CNQX and NBQX have become the standard antagonists for inhibition of neuronal AMPA receptors. Our results, together with those of other recent studies (7, 22, 37), indicate that these compounds also produce potent inhibition at kainate receptors. Compared with the noncompetitive 2,3-benzodiazepine GYKI 53655, which shows >200-fold higher affinity at AMPA than at kainate receptors (20, 21), even NBQX displayed only a 3-fold difference in potency for inhibition of whole-cell currents. Although previous work (26, 44) demonstrated relatively strong selectivity for quinoxalinediones between high affinity AMPA and kainate binding sites, recent analysis of cloned receptor subunits suggests that functional ion channels correspond to subunit combinations with lower affinity for kainate (K_d = 50–100 nM), which include the GluR5 or GluR6 subunit (12). High affinity kainate binding sites (K_d for kainate = 5–10 nM), formed by expression of the KA1 or KA2 subunits in isolation, do not make functional channels (12).

In the current study, we determined antagonist potency against kainate receptors in DRG neurons that are thought to include the GluR5 and KA2 subunits (2, 6, 8). Recent work on homomeric GluR6 receptors expressed in 293 cells revealed similar potencies for CNQX and NS-102 in that system (22). However, a study of kainate receptors in embryonic hippocampal neurons (40), which are thought to express the GluR6 and, in some cells, GluR5 subunit (45), found NS-102 to be significantly more potent than CNQX. Clearly, further work will be needed to determine whether the various antagonists used in this study show similar potency against kainate receptors resulting from other subunit combinations that may be expressed by other cells in the nervous system. Nevertheless, in the case of DRG cells, our results emphasize the broad overlap in potency of competitive antagonists at kainate receptors and CNS AMPA receptors.

Acknowledgments

We are grateful to ACEA Pharmaceuticals, Marion Merrell Dow, Merck Sharp and Dohme, NeuroSearch, and Novo Nordisk for providing compounds used in this study and to Mike Finley for critical comments on the manuscript.

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