Species-dependent functional properties of non-NMDA receptors expressed in *Xenopus laevis* oocytes injected with mammalian and avian brain mRNA

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1 Species-dependent variation in the functional properties of non-NMDA receptors was investigated by intracellular recording in *Xenopus laevis* oocytes injected with rat, chick and calf brain mRNA.

2 In all mRNA-injected oocytes, kainic acid (KA), domoic acid (Dom) and 5-bromowillardiine (BrW) evoked large, maintained membrane currents, in contrast to the smaller, desensitizing responses elicited by α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), quisqualic acid (QA) and L-glutamic acid (L-Glu). Dose-response curves for KA in oocytes injected with calf ($EC_{50} = 96.4 \pm 12.3 \mu\text{M}$; mean $\pm$ s.e.mean), chick ($87.0 \pm 8.9 \mu\text{M}$) or rat ($88.7 \pm 4.3 \mu\text{M}$) brain mRNA were similar.

3 Current-voltage ($I-V$) relationships determined with KA inwardly rectified in oocytes injected with calf or chick mRNA; whereas, outward rectification was observed in oocytes injected with rat brain mRNA.

4 In oocytes injected with rat brain mRNA, AMPA antagonized responses evoked by KA in a competitive manner. The absolute amplitudes of KA and AMPA responses in the same oocytes were significantly correlated, which is consistent with both agonists acting on the same receptor-ionophore complex.

5 In contrast, in oocytes injected with calf or chick brain mRNA, AMPA (QA and L-Glu) antagonized the response evoked by KA in a non-competitive manner. The response amplitudes of KA compared to AMPA, QA or L-Glu in the same oocytes were not correlated suggesting discrete receptor-ionophores.

6 This study favours the existence of distinct non-NMDA receptor subtypes that are equi-sensitive to KA. The expressed receptors from different species of mRNA may be distinguished by their voltage-sensitivities and the type of antagonism exerted by AMPA on KA-activated responses. Our observations may reflect further heterogeneity of non-NMDA receptors in the central nervous system of different vertebrate species.

Keywords: Kainate; intracellular recording; glutamate receptor heterogeneity; mRNA translation; *Xenopus laevis* oocyte

Introduction

In the central nervous system (CNS), heterogeneity amongst neurotransmitter receptor subtypes has been established as a general phenomenon from radioligand binding, electrophysiological and molecular cloning studies. For non-N-methyl-D-aspartate (non-NMDA) receptors, multiple cDNAs coding for distinct receptor subunits in the rat exist which display diverse functional properties (Gasic & Hollmann, 1992; Sommer & Seeburg, 1992; Barnes & Henley, 1992). In some cases, correlations between the functional properties of recombinant and native neuronal non-NMDA receptors are evident. For example, high (Robinson & Deadwyler, 1981; Ben-Ari & Gho, 1988) and low (Perouansky & Grantyn, 1989; Patneau & Mayer, 1990) binding affinities at neuronal non-NMDA receptors by kainate (KA) have also been noted on recombinant receptors (Wisden & Seeburg, 1993). In addition, KA-induced membrane currents in neurones can exhibit inward (Iino *et al.*, 1990; Gilbertson *et al.*, 1991) or outward (Mayer & Westbrook, 1984) rectification. Similar rectifying behaviour has been observed with recombinant non-NMDA receptors (Boulter *et al.*, 1990; Verdoorn *et al.*, 1991). In these studies, membrane currents induced on receptors comprising either GluR1 and/or GluR3 subunits, possessed inwardly rectifying properties. Co-expression of GluR1 or GluR3 with GluR2 formed heteromeric ion channels devoid of inward rectification.

Although most neuronal or cloning studies have been per-

formed in the rat, it is assumed, by analogy, that a similar functional heterogeneity exists for non-NMDA receptors in other vertebrate species. In view of this, the present investigation was initiated to determine whether non-NMDA receptors from different vertebrate species possess similar functional properties to native receptors in the rat. For this, the *Xenopus laevis* oocyte was used to compare the functional properties of non-NMDA receptors expressed from calf and chick brain mRNA with those expressed from rat brain mRNA. Our results suggest that receptor heterogeneity can occur between different vertebrate species. Preliminary accounts of this work have been published previously in abstract form (Bowie & Smart, 1989; 1990; 1991b).

Methods

Extraction of messenger RNA

Messenger ribonucleic acid (mRNA) was extracted from 3-month old calf brain cortices, 1 day old chick whole brains and 3–6 week old rat whole brains (all devoid of the cerebellum) using a guanidinium thiocyanate/caesium chloride method (Chirgwin *et al.*, 1979). The poly (A)-mRNA was isolated by oligo-(dT)-cellulose chromatography, then resuspended in water at 1 mg ml^{-1} and stored at -70°C .

Preparation of oocytes and microinjection with mRNA

Mature female *Xenopus laevis* toads were anaesthetized by immersion in a cold solution of 0.2% w/v ethyl-*m*-amino-

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benzoate (tricaine) for 15–30 min. Part of an ovary containing up to 100–200 oocytes was excised and transferred directly into a modified Barth's solution (MBS) containing (mM): NaCl 110, KCl 1, NaHCO₃ 2.4, Tris/HCl 7.5, Ca(NO₃)₂ 0.33, CaCl₂ 0.41, MgSO₄ 0.82 streptomycin 10 mg ml⁻¹, pH 7.6. Healthy oocytes at stages V and VI were preferentially selected for injection. Each oocyte, with an intact follicular layer, was injected with approximately 50 nl of mRNA solution (1 mg ml⁻¹) into the equatorial region. Injected oocytes were transferred into fresh MBS and incubated at 17–18°C for 2–3 days to permit the translation of mRNA. The MBS was replaced on alternate days. Once the oocytes became responsive to the test agonists they were then stored at 10°C which prolonged their survival for up to 4–5 weeks. Under these conditions, the survival of injected and uninjected oocytes were almost the same. In the first 5 days *in vitro*, 83.1 ± 2.5% (1496 oocytes, 18 batches, 4 donors) of follicular oocytes survived after being injected with calf brain mRNA. In comparison, 80 ± 11% (640 oocytes, 8 batches, 3 donors) of uninjected oocytes survived over the same period. The survival of injected oocytes did not apparently vary throughout the year which was monitored consecutively over a 3 year period.

Electrophysiology

Electrophysiological recordings were performed in a Perspex recording chamber (adjustable volume: 0.15–1 ml) lined with Sylgard and superfused at 8–10 ml min⁻¹ with an amphibian Ringer containing (mM): NaCl 110, KCl 2, HEPES 5, CaCl₂ 1.8, pH 7.4. Oocytes were impaled with two microelectrodes manufactured from thin-walled borosilicate glass and filled with 3 M KCl, providing resistances of 5–10 MΩ for the voltage-recording and 1–2 MΩ for the current-injecting microelectrodes. All electrophysiological experiments were performed using an Axoclamp 2A in two electrode voltage clamp recording mode. Only oocytes with a membrane input resistance (R_{IN}) and membrane potential (E_m) of 1–5 MΩ and –40 to –60 mV respectively were accepted for experimentation. Experiments were performed at room temperature and drugs were dissolved in Ringer and bath-applied.

Calculation of membrane conductance

The membrane potential was voltage-clamped at a holding potential (V_H) of –50 mV to –60 mV. The membrane conductance evoked by the agonist was determined by subtraction after applying voltage command steps in the presence and absence of the agonist. Membrane conductance data were used to construct dose-response curves which were fitted using a logistic equation (see Figure 1 legend) employing a Marquadt non-linear least squares routine.

Determination of agonist current-voltage relationships

Current-voltage ($I-V$) relationships were studied in voltage clamped oocytes by stepping the holding potential (V_H) = –40 mV to –60 mV with voltage command steps (20 mV increments, 1.5 s duration, applied every 20 s) in the presence and absence of agonist. Instantaneous currents were measured after the command step achieved a steady state (usually within 10 ms). The $I-V$ s were only determined in the presence of agonist after the agonist-evoked membrane current attained a steady-state. The agonist $I-V$ was determined by subtracting the control $I-V$ from the $I-V$ determined in the presence of the agonist. To determine the voltage sensitivity of the agonist-induced conductances, the membrane chord conductances evoked by agonists at different membrane potentials were calculated using the equation,

$$\text{Chord } G = I/(E_m - V_{rev})$$

where chord G and I are the chord conductance and membrane current evoked by the agonist, E_m represents the mem-

brane potential and V_{rev} the agonist reversal potential. The $I-V$ relationship evoked by KA in *Xenopus laevis* oocytes can be contaminated by a chloride conductance ($I_{Cl(Ca^{2+})}$) which is activated by calcium entering the cell through non-NMDA ion channels. We have previously shown in oocytes injected with calf and chick brain mRNA that activation of $I_{Cl(Ca^{2+})}$ influences the $I-V$ relationship to KA producing outward rectification at positive membrane potentials (Bowie & Smart, 1993a). Therefore, to ensure the agonist $I-V$ s were not contaminated by $I_{Cl(Ca^{2+})}$, $I-V$ s were also constructed in the presence of the chloride channel blocker, anthracene-9-carboxylic acid (1 mM A9C). In the present study, all the $I-V$ s were unaffected by the inclusion of A9C suggesting that $I_{Cl(Ca^{2+})}$ did not contaminate $I-V$ plots determined for non-NMDA receptor agonists.

Drugs

(RS)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), quisqualic acid (QA), ($\pm$)-willardiine and 5-bromo-willardiine (BrW) were obtained from Tocris Neuramin. Kainic acid (KA) and L-glutamic acid (L-Glu) were obtained from Sigma and Professor E.A. Barnard kindly supplied domoic acid.

Results

Two types of response for non-NMDA receptor agonists

In oocytes injected with any of the different mRNA species, KA (80 μ M; $n = 182$) produced a large, apparently non-desensitizing response which was associated with an increase in membrane conductance. In contrast, the responses evoked by AMPA (100 μ M; $n = 40$) were much smaller and showed signs of desensitization (Figure 1a). To highlight these differences, the peak response of a variety of non-NMDA receptor agonists were compared in 50 oocytes injected with calf brain mRNA. Similar to 80 μ M KA (51 ± 5 nA, $n = 27$), the responses evoked by 20 μ M Dom (59 ± 7 nA, $n = 6$) and 20 μ M BrW (56 ± 10 nA, $n = 7$) were large and non-desensitizing. However, membrane currents evoked by 6 μ M QA (4 ± 0.7 nA, $n = 13$), 50 μ M L-Glu (5.7 ± 0.9 nA, $n = 11$) and 100 μ M AMPA (3.2 ± 0.6 nA, $n = 11$) were smaller and desensitizing. Similar observations were also made on oocytes injected with chick and rat brain mRNA.

Equilibrium dose-response curves to kainate

Equilibrium dose-response curves were constructed to KA to make a direct quantitative comparison between non-NMDA receptors expressed from different vertebrate species. The expressed non-NMDA receptors showed a similar degree of sensitivity to KA in oocytes injected with calf, chick or rat brain mRNA where the estimated EC₅₀ values were 96.4 ± 12.3 μ M ($n = 20$), 87.0 ± 8.9 μ M ($n = 6$) and 88.7 ± 4.3 μ M ($n = 5$) respectively (Figure 1b).

Current-voltage relationships to kainate

Two distinct types of current-voltage ($I-V$) relationship for KA were revealed in oocytes injected with different mRNA species. With calf or chick brain mRNA, the membrane current evoked by 80 μ M KA exhibited pronounced inward rectification at positive membrane potentials. In contrast, the membrane current evoked by 80 μ M KA in oocytes injected with rat brain mRNA showed only slight outward rectification (Figure 2a). The voltage sensitivity of the two types of KA response was further demonstrated by determining the chord conductance-voltage ($G-V$) relationships (Figure 2b). In oocytes injected with calf ($n = 4$) or chick ($n = 3$) brain mRNA, KA (80 μ M) produced a bell-shaped chord $G-V$ plot which described a region of maximum membrane conduc-

tance between -60 to -100 mV membrane potential (Figure 2b). The minimum membrane conductance was approached at positive membrane potentials (i.e. $> +10$ mV). In comparison, the membrane conductance produced by $80 \mu\text{M}$ KA ($n = 6$) in oocytes injected with rat brain mRNA increased at depolarised potentials greater than $+20$ mV, but was rela-

tively voltage-insensitive at membrane potentials more negative than -20 mV (Figure 2b).

The voltage-dependency of the response evoked by a variety of non-NMDA receptor agonists and at different agonist concentrations were compared using a chord conductance ratio (G_R) (Table 1; cf. Bowie & Smart, 1991a). The non-NMDA receptor agonists, BrW and Dom, evoked responses which exhibited the same type of voltage-dependency to that observed with KA. For example, the G_R value for $80 \mu\text{M}$ KA ($G_R = 0.45 \pm 0.06$, $n = 7$) in oocytes injected with chick brain mRNA was similar to that determined for equi-effective concentrations of Dom ($20 \mu\text{M}$; 0.51 ± 0.05 , $n = 4$) and BrW ($20 \mu\text{M}$; 0.47 ± 0.06 , $n = 5$) (Table 1). Similar observations were made in oocytes injected with calf brain mRNA (Table 1). In addition, the degree of rectification observed in oocytes injected with calf or chick brain mRNA was dependent on the agonist concentration (Table 1). A comparison of the G_R values calculated for KA (20 – $160 \mu\text{M}$) revealed that the ability of the KA-activated channel to pass outward current was further compromised at increasing agonist concentrations (Figure 2c; see also Table 1). An example of a series of I – V plots in the same oocyte determined with different concentrations of KA is shown in Figure 2c. The rectification was not dependent on the absolute amplitude of the membrane current evoked by the agonists in each oocyte, since similar G_R values were observed at a given agonist concentration in oocytes exhibiting markedly different levels of receptor expression. In contrast to oocytes injected with calf or chick brain mRNA, the voltage-sensitivity of the response evoked by KA in oocytes injected with rat brain mRNA was apparently independent of agonist concentration (Table 1).

Interactions between kainate and other non-NMDA receptor agonists

The different I – V relationships observed to KA may be a consequence of several distinct receptor-channel complexes activated only by KA. This possibility was examined by studying the interaction of KA with AMPA, QA and L-Glu on non-NMDA receptors expressed from the different species of mRNA. In all mRNA-injected oocytes ($n = 52$), near saturating concentrations of QA ($6 \mu\text{M}$), AMPA ($100 \mu\text{M}$) and L-Glu ($50 \mu\text{M}$) reduced the membrane current evoked by $80 \mu\text{M}$ KA. For example, AMPA ($100 \mu\text{M}$) reduced the response to $80 \mu\text{M}$ KA by $55 \pm 5\%$ ($n = 5$), $47 \pm 4\%$ ($n = 4$) and $54 \pm 2\%$ ($n = 10$) in oocytes injected with calf, chick and rat brain mRNA respectively. The antagonism of the KA-induced response by preincubation or co-application of QA, AMPA or L-Glu had a rapid onset and recovery. Furthermore, the antagonism did not show any use-dependency and was fully reversible. Responses to other agonists, such as BrW and Dom were similarly antagonized by QA. In oocytes injected with calf brain mRNA, the order of potency for inhibition of the KA-activated response, assessed from the IC_{50} s, was: QA ($IC_{50} = 5 \mu\text{M}$) $\gg$ L-Glu ($30 \mu\text{M}$) $>$ AMPA ($50 \mu\text{M}$) ($n = 4$).

Figure 1 Membrane current profiles and concentration-response relationships for non-NMDA receptor agonists in mRNA-injected oocytes. (a) Typical membrane currents (holding potential, V_H -60 mV) evoked by $80 \mu\text{M}$ kainic acid (KA) and $200 \mu\text{M}$ α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) in an oocyte injected with calf brain mRNA. Negative voltage command steps (-5 to -10 mV, 1.5 s duration, 0.05 Hz) were used to monitor the membrane conductance. Agonists were applied for the duration indicated by the solid line. (b) Equilibrium dose-response curves constructed for KA applied to oocytes injected with calf ($\circ$, $n = 20$), rat (Δ , $n = 5$) or chick ($\square$, $n = 6$) brain mRNA. The membrane conductances induced by KA were normalized in each dose-response curve to the response evoked by $80 \mu\text{M}$ KA and expressed as the mean $\pm$ s.e.mean (ΔG_N). The curves were fitted by a logistic function of the form, $I/I_{\max} = 1/(1 + (EC_{50}/[A])^n)$, where I and $I_{\max}$ represent the agonist-induced current at a given concentration and the maximum current induced by a saturating concentration of agonist. EC_{50} defines the concentration of agonist ($[A]$) which induces 50% of the maximum response and n , is the Hill coefficient. The following estimates of EC_{50} s and n were obtained for KA; calf brain mRNA, $EC_{50} = 96.4 \pm 12.3 \mu\text{M}$, $n = 1.42 \pm 0.2$; chick brain mRNA, $EC_{50} = 87.0 \pm 8.9 \mu\text{M}$, $n = 1.36 \pm 0.1$; rat brain mRNA, $EC_{50} = 88.7 \pm 4.3 \mu\text{M}$, $n = 1.78 \pm 0.1$. To avoid receptor desensitization, dose-response curves were not constructed by applying high and low agonist concentrations alternately and supramaximal concentrations of KA ($> 640 \mu\text{M}$) were avoided.

Table 1 Mean chord conductance rectification ratios (G_R) for non-NMDA receptor agonists calculated from individual I – V relationships determined in calf, chick and rat brain mRNA-injected oocytes

Agonist	Calf	Chick	Rat
KA $20 \mu\text{M}$	0.81 ± 0.16 (4)	0.78 ± 0.14 (3)	1.42 ± 0.19 (3)
KA $80 \mu\text{M}$	0.44 ± 0.06 (9)	0.45 ± 0.06 (7)	1.49 ± 0.15 (6)
Dom $20 \mu\text{M}$	0.55 ± 0.04 (5)	0.51 ± 0.05 (4)	ND
BrW $20 \mu\text{M}$	0.52 ± 0.08 (3)	0.47 ± 0.06 (5)	ND

G_R was determined by calculating the chord conductances at $+30$ mV and -50 mV and using these values to define the ratio ($G_R = G(+30)/G(-50)$). All agonists tested had similar reversal potentials ($V_{\text{rev}} = -6 \pm 2$ mV, $n = 72$). G_R values less than one were associated with an I – V relationship which displayed inward rectification, whereas values greater than one predicted outward rectification. The values in parentheses indicate the number of oocytes examined. All values are mean $\pm$ s.e.mean; ND: not determined. KA: kainic acid; Dom: domoic acid; BrW: 5-bromowillardiine.

Figure 2 Current voltage ($I-V$) relationships for kainic acid (KA)-activated responses. (a) $I-V$ s were determined in oocytes injected with either calf (O), chick (□) or rat brain (Δ) mRNA ($V_H = -60$ mV). For comparison, the agonist-evoked currents at different membrane potentials were normalized to the current induced by 80 μM KA in each oocyte at -60 mV. KA characteristically produced inward rectification at membrane potentials near to 0 mV in oocytes injected with calf or chick brain mRNA. The membrane current evoked by KA in the oocyte injected with rat brain mRNA exhibited outward rectification. The curves fitted to data points in the $I-V$ s were generated from third order polynomials. These equations were used to calculate the reversal potentials and chord conductances. (b) Chord conductance-membrane potential ($G-V$) relationships for kainate. $I-V$ relationships to 80 μM KA were used to generate the $G-V$ plots (see Methods). For comparison, the agonist-evoked chord conductances were normalized to the chord conductance obtained at -40 mV membrane potential (G_N). Normalised $G-V$ relationships were obtained for KA using oocytes injected with rat (Δ, $n = 6$), calf (O, $n = 4$) or chick (□, $n = 3$) brain mRNA. (c) $I-V$ relationships at different concentrations of KA

Competitive and non-competitive antagonism of kainate responses

In oocytes injected with rat brain mRNA, AMPA 100 μM produced an apparent parallel shift of the KA dose-response curve without any modification to the maximum KA-induced response (Figure 3a). The estimated EC_{50} values for KA in the absence and presence of AMPA were 97 μM and 327 μM respectively. These observations are consistent with competitive antagonism and indicated that both agonists are probably binding to the same receptor-ionophore complex.

Figure 3 Competitive interaction of kainic acid (KA) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) on expressed non-NMDA receptors. (a) Equilibrium dose-response curves for KA-induced membrane conductance (G) in the absence (O) and presence (●) of 100 μM AMPA determined in an oocyte injected with rat brain mRNA at $V_H = -60$ mV. The curves were fitted to the data points using a logistic function providing the following estimates of the EC_{50} and n for the control curve, 97 ± 6 μM and 1.5 ± 0.1 ; and in the presence of AMPA, 327 ± 26 μM and 1.2 ± 0.1 respectively. (b) The amplitude of membrane currents ($V_H = -60$ mV) evoked by 80 μM KA (I_{KA}) were compared with currents induced by 100 μM AMPA (I_{AMPA}) in the same rat brain mRNA-injected oocytes. The correlation was assessed using unweighted linear regression with $r = 0.9785$, $t = 13.4$ and $P = 9.16E-7$.

(20 μM–160 μM) were determined in the same oocyte injected with calf brain mRNA ($V_H = -50$ mV). Increasing KA concentration apparently increased the rectification for 20 μM (O), 40 μM (●), 80 μM (□) and 160 μM (■) KA where the G_R values are 0.81 ± 0.10 , 0.70 ± 0.08 , 0.43 ± 0.11 and 0.35 ± 0.02 respectively ($n = 4$). Curve fits were generated from fourth order polynomials.

Figure 4 Apparent non-competitive interaction of kainic acid (KA) and quisqualic acid (QA) on expressed non-NMDA receptors. (a) Equilibrium dose-response curves for normalised conductances (ΔG_N) induced by KA in the absence (○) and presence (●) of 6 μM QA determined in oocytes injected with calf brain mRNA ($n = 6$; $V_H = -60$ mV). KA-activated responses were normalized to the control 80 μM KA response and expressed as the mean $\pm$ s.e.mean. The logistic function was used to fit curves to the data points providing the following estimates of EC_{50} and n ; control, 103 ± 5 μM and 1.7 ± 0.1 ; and in the presence of QA 222 ± 19 μM , 1.2 ± 0.1 . (b and c) The amplitudes of membrane currents evoked by 80 μM KA were compared at -60 mV with those induced (I_{Agonist}) by 6 μM QA (○), 100 μM AMPA (●) and 50 μM L-glutamic acid (L-Glu, □) in the same oocytes injected with either chick (b; $n = 16$) or calf (c; $n = 36$) brain mRNA. Correlations were assessed between I_{KA} and I_{Agonist} using unweighted linear least squares analyses providing the following values for chick brain mRNA: I_{QA} , $r = 0.6023$, $t = 1.6872$; I_{AMPA} , $r = 0.4541$, $t = 1.0196$; and I_{Glu} , $r = -0.6698$, $t = -0.9021$. For calf brain mRNA: I_{QA} , $r = 0.4280$, $t = 1.7719$; I_{AMPA} , $r = 0.5463$, $t = 1.9565$; and I_{Glu} , $r = 0.5953$, $t = 2.0955$. All correlations were insignificant at the 5% level. (d) In an oocyte injected with calf brain mRNA, responses were evoked to 80 μM KA, 6 μM QA and 80 μM KA + 6 μM QA in control Ringer and after incubation with 10 μM concanavalin A (Con-A) for 30 min. Con-A enhanced the response evoked by QA; however, the antagonism of the KA response by QA was unaffected (54% in control Ringer, 56% with Con-A). The 25 nA calibration applies to the traces for 6 μM QA.

This notion was supported by the significant correlation ($P < 0.001$) between the membrane current amplitudes evoked by KA (80 μM) and AMPA (100 μM) in the same oocytes, reflecting the relative level of receptor expression (Figure 3b). All the oocytes injected with rat brain mRNA ($n = 9$) consistently exhibited a sensitivity to both KA and AMPA.

In contrast, in oocytes injected with either calf or chick brain mRNA, QA or AMPA antagonized the KA dose-response curve in an apparently non-competitive manner. The equilibrium dose-response curve constructed for KA ($n = 6$, calf mRNA) in the presence of QA (6 μM) was shifted slightly to the right with a clear reduction in the maximum response to KA (Figure 4a). The estimated EC_{50} values for KA in the absence and presence of QA were 103 μM and 222 μM respectively. At low concentrations of KA ($< 20 \mu\text{M}$), the membrane current evoked by combinations of KA and QA was larger than that recorded to KA alone. In this case, the net response observed during co-application of these agonists was the summation of their individual responses.

Interestingly, unlike rat brain mRNA-injected oocytes, a comparison of the membrane current amplitudes evoked by 80 μM KA with currents evoked by QA (6 μM , $n = 22$), AMPA (100 μM , $n = 17$) or L-Glu (50 μM , $n = 13$) in the same oocytes injected with chick ($n = 16$; Figure 4b) or calf ($n = 36$; Figure 4c) brain mRNA were not significantly correlated ($P > 0.05$). This result is apparently inconsistent with the agonists sharing the same receptor but could be accounted for by assuming discrete receptor-ionophore complexes. Moreover, in some oocytes, a response to KA could be observed in the absence of any measurable QA response; even at concentrations of QA which would be regarded as saturating. In these QA-insensitive oocytes ($n = 3$), the response to KA was still antagonized by QA.

To test the possibility that the absence of measurable QA-responses was due to a rapid and full receptor desensitization induced by QA, the plant lectin, concanavalin-A (Con-A), was used to reduce receptor desensitization (Mayer & Vyklícky, 1989). Figure 4d shows a typical example of the effect of Con-A (10 μM) on the antagonism of KA (80 μM) responses by 6 μM QA. In oocytes injected with calf ($n = 4$) or chick ($n = 3$) brain mRNA, Con-A increased the response to QA; however, the reduction of the KA-response by QA following Con-A treatment was similar to the inhibition in control Ringer. This suggested that the level of Con-A-sensitive receptor desensitization does not affect the degree of antagonism.

Discussion

Our primary objective in this study was to examine the possibility of species-dependent variation in the functional properties of non-NMDA receptors expressed from three vertebrate species. The *Xenopus laevis* oocyte was used since it was possible to study each expressed non-NMDA receptor in the same membrane environment. In this study, non-NMDA receptors expressed from rat brain mRNA were apparently activated by both KA and AMPA and possessed similar properties to previously described native neuronal and expressed recombinant glutamate receptors derived from the rat. In oocytes injected with calf and chick brain mRNA, our data were compatible with the existence of distinct non-NMDA receptors activated by KA and AMPA. As yet, non-NMDA receptors from calf and chick have not been cloned nor has there been any extensive electrophysiological studies of neurones derived from these species. Non-NMDA receptors expressed from calf and chick brain mRNA are typified by a novel non-competitive antagonism of KA responses by AMPA, QA or L-Glu. We propose that these observations may reflect further heterogeneity of non-NMDA receptor subtypes present in the CNS of a variety of vertebrates.

Expression of non-NMDA receptors equi-sensitive to kainate

The mRNAs in this study have been extracted from brain tissue which includes a variety of cell types of neuronal, glial, ependymal and vascular origin. Neurones are a favourable candidate for the source of mRNA since, in addition to their large number in the CNS, the receptors expressed in this study displayed similar properties to their neuronal counterparts. For example, expressed receptors from different species were equi-sensitive to KA with a similar potency to that determined in mammalian (Perouansky & Grantyn, 1989; Patneau & Mayer, 1990), avian (O'Brien & Fischbach, 1986; Zorumski & Yang, 1988) and fish (Ishida & Neyton, 1985) neurones. In addition, the different $I-V$ relationships determined for KA in this study have also been observed in neuronal studies (Ozawa *et al.*, 1991). However, non-NMDA receptors are not solely confined to neurones since glial cells, which may outnumber neurones by 10:1 (Kuffler *et al.*, 1984), also respond to excitatory amino acid receptor agonists (Usowicz *et al.*, 1989; Wyllie *et al.*, 1991; Muller *et al.*, 1992; Burnashev *et al.*, 1992). Although non-NMDA receptors expressed by glial cells have not yet been characterized as thoroughly as their neuronal counterparts, electrophysiological studies have revealed similar pharmacological and biophysical properties (Usowicz *et al.*, 1989; Wyllie *et al.*, 1991; Muller *et al.*, 1992; Burnashev *et al.*, 1992). In addition, it was also difficult to compare the receptor properties between species at the same developmental stage and thus eliminate the possibility that our data may be explained by a differential age-dependent expression of glutamate receptor subunits.

Interaction of KA and AMPA with non-NMDA receptors

In neurones or mRNA-injected oocytes, the responses to KA can be antagonized by other non-NMDA agonists in either a competitive or non-competitive manner. Usually, the antagonism was competitive and could be relieved by increasing the concentration of KA (O'Brien & Fischbach, 1986; Perouansky & Grantyn, 1989; Patneau & Mayer, 1991; Thio *et al.*, 1991; Bowie & Smart, 1993b), as also observed for the AMPA inhibition of KA-induced responses in oocytes injected with rat brain mRNA. This is consistent with a common receptor-ionophore for KA and AMPA, supported by the correlation between the amplitudes of KA and AMPA-induced responses measured in the same oocytes. It is not known whether KA and AMPA bind to the same agonist recognition site on the receptor-ionophore complex or if discrete binding sites exist (cf. Patneau & Mayer, 1991). However, thiocyanate ions can pharmacologically distinguish between KA- and AMPA-evoked responses on the same receptor ionophore complex which would favour discrete agonist binding sites (Bowie & Smart, 1993b).

Rarely, the antagonism of KA responses by QA (AMPA or L-Glu) was non-competitive (Ishida & Neyton, 1985; Aizenman *et al.*, 1988; Bowie & Smart, 1990; 1991b). This antagonism could also occur in the absence of any response to QA, favouring distinct receptor-channel complexes for KA and QA (or AMPA) (Ishida & Neyton, 1985; Aizenman *et al.*, 1988; Bowie & Smart, 1990; 1991b). However, Thio *et al.* (1991) observed non-competitive antagonism of KA-induced responses by QA on rat hippocampal neurones and concluded that both non-NMDA receptor agonists activated a single receptor-channel complex. Non-competitive antagonism was only observed when QA was applied before KA. QA was assumed to desensitize some of the non-NMDA receptors, since wheat germ agglutinin abolished the antagonism. Although in our study, non-competitive antagonism of KA responses was observed when QA was pre- or co-applied with KA, we cannot discount the possibility that in some oocytes, QA may induce a rapid and full desensitization of

non-NMDA receptors. This could have three consequences, firstly, QA-activated responses may no longer be resolved; secondly, QA could still compete for a common binding site with KA thereby acting as an antagonist; and thirdly, the desensitization may cause a reduction in the maximum response evoked by KA. Cross desensitization between QA or L-Glu and KA-activated responses has been proposed to account for agonist interactions on the same receptor-ionophore in hippocampal pyramidal neurones (Kiskin *et al.*, 1986; 1990; Thio *et al.*, 1991). However, in the present study, a reduction in the level of QA-induced desensitization by Con-A did not affect the antagonism of KA-induced responses (Figure 4d; cf. Mayer & Vyklicky, 1989). An alternative explanation is that QA desensitizes the receptor by additional Con-A insensitive mechanisms.

The failure to correlate KA- and QA- (AMPA or L-Glu) induced response amplitudes in the same oocytes injected with calf or chick brain mRNA is consistent with the activation of discrete receptor ionophores. However, if the degree of desensitization induced by QA varies between oocytes, this would account for the lack of any correlation in response amplitudes. A less likely hypothesis to explain our data would involve a direct occlusion of KA-activated ion channels by QA, AMPA or L-Glu. This has been discounted since the voltage-dependency expected for this type of antagonism was not observed (Bowie, 1991).

A more complex model compatible with our results, involves the allosteric modulation by QA (AMPA or L-Glu) of a receptor sensitive to KA, causing a conformational change in the receptor channel complex to a non-conducting state. In this model, QA binds to two sites; a receptor specific for this agonist and an allosteric site where QA acts only as an antagonist. The ability of QA to antagonize responses to KA in the absence of any measurable agonist response to QA and the lack of any correlation between the amplitudes of QA and KA responses are both consistent with QA acting at an allosteric site on a KA receptor. It is an interesting correlation that non-competitive antagonism was only observed on non-NMDA receptors which mediate voltage-sensitive KA-activated responses.

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Heterogeneity amongst non-NMDA receptors activated by kainate

The functionally diverse properties of expressed non-NMDA receptors from different species, may be accounted for by assuming the existence of a number of distinct non-NMDA receptors, with equal sensitivity to KA. In the CNS of a variety of species, heterogeneous populations of non-NMDA receptors may exist which are selective for QA and AMPA (Aizenman *et al.*, 1989; Henley & Barnard, 1989b; Yoneda & Ogita, 1989), KA and AMPA (Honore & Drejer, 1988; Henley & Barnard, 1989a; Henley *et al.*, 1989) or KA alone (Agrawal & Evans, 1986). The physiological significance of non-NMDA receptor heterogeneity has not been fully resolved. Ozawa *et al.* (1991) have suggested that different types of KA-receptor may be associated with particular neurones since inward rectification was observed in smaller hippocampal neurones whereas outward rectification was found in larger cells that were probably young pyramidal neurones; although, inward rectifying excitatory synaptic currents in hippocampal interneurones are not associated with any particular cellular morphology (McBain & Dingledine, 1993). More recent studies have suggested a further complexity since more than one type of non-NMDA receptor may be expressed by a single neurone (Wyllie *et al.*, 1993) and that each receptor may have selective subcellular localization (Craig *et al.*, 1993). In view of this, one possible role for receptor heterogeneity (e.g. at central synapses) may be to provide a subtle modification of the neurotransmitter response through the expression of a mosaic of several non-NMDA receptor subtypes.

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