Br. J. Pharmacol. (1993), **109**, 779–787

Thiocyanate ions selectively antagonize AMPA-evoked responses in *Xenopus laevis* oocytes microinjected with rat brain mRNA

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1 Responses to kainate (KA), willardiine and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) were recorded from rat brain mRNA-injected *Xenopus laevis* oocytes by use of a two-electrode voltage clamp.

2 Thiocyanate (SCN^-; 50 μM–4 mM) ions reversibly and selectively inhibited the membrane current responses to AMPA in a non-competitive manner without affecting KA or willardiine-induced responses.

3 The inhibition of AMPA-induced responses by SCN^- was dependent on the SCN^- concentration with an estimated IC_{50} of 1 mM. The antagonism was not dependent on the AMPA concentration.

4 The response to a high concentration of AMPA (100–200 μM) exhibited a peak inward current which declined to a steady-state. SCN^- inhibited the steady-state current more than the peak response. The inhibition was unaffected by prior incubation with concanavalin-A (Con-A; 10 μM).

5 Responses to KA were antagonized by AMPA in a competitive manner, suggesting that both agonists may activate a common receptor-channel complex. This interaction between two non-NMDA agonists was not affected by the SCN^--induced inhibition of the AMPA response.

6 AMPA-induced responses recorded from large cultured cerebellar neurones by whole-cell recording were also inhibited by SCN^- in a non-competitive manner. The AMPA-induced peak current was less affected than the steady-state response.

7 We conclude that SCN^- can inhibit the response to AMPA in expressed non-NMDA receptors in *Xenopus* oocytes and also in native receptors in cultured cerebellar neurones. One possible mechanism of action for SCN^- inhibition of responses to AMPA may involve a Con-A-insensitive, non-NMDA receptor-mediated desensitization.

Keywords: Non-NMDA receptors; AMPA; thiocyanate; *Xenopus laevis*; intracellular recording; mRNA translation; cerebellar neurones; patch clamp

Introduction

In radioligand binding and electrophysiological studies, a variety of cations have been shown to modulate the properties of excitatory amino acid (EAA) receptors. For example, at N-methyl-D-aspartate (NMDA) receptors, both Mg^{2+} and Zn^{2+} can act as non-competitive antagonists (Mayer *et al.*, 1984; Nowak *et al.*, 1984; Peters *et al.*, 1987; Westbrook & Mayer, 1987; Enomoto *et al.*, 1992) by binding to discrete sites on the NMDA receptor-channel complex (Mayer *et al.*, 1988). In addition, modulation of the external pH by changing the H^+ ion concentration also inhibited the activity of NMDA receptors in a non-competitive manner probably by binding to another discrete site (Tang *et al.*, 1990; Vyklicky *et al.*, 1990; Traynelis & Cull-Candy, 1990; 1991). As all these ions are naturally present *in vivo* in the central nervous system (CNS), they may be capable of regulating the activity of one or more members of the NMDA receptor family under physiological or even pathological conditions (cf. Forsythe *et al.*, 1988; McDonald & Johnston, 1990).

In comparison, non-NMDA receptors are not apparently regulated by endogenous substances which are so vital for NMDA receptor modulation, such as glycine (Johnson & Ascher, 1987) and Mg^{2+} (Nowak *et al.*, 1984; Mayer *et al.*, 1984). Nevertheless responses evoked by kainate (KA) can be non-competitively depressed by divalent cations such as Hg^{2+} (Kiskin *et al.*, 1989; Umbach & Gundersen, 1989) or Ca^{2+} (Perouansky & Grantyn, 1989; Gu & Huang, 1991). Moreover, in binding studies, Ca^{2+} is the most potent cation to depress [^{3}H]-KA binding (Honore *et al.*, 1986; Monaghan *et al.*, 1986) and could be used to distinguish selectively between KA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) binding to non-NMDA receptors.

As with NMDA receptors, lowering the external pH also reduced non-NMDA receptor-mediated function (Randle *et al.*, 1988; Christensen & Hida, 1990; Vyklicky *et al.*, 1990; Traynelis & Cull-Candy, 1991). However, in comparison to NMDA receptors, Zn^{2+} ions could either enhance or inhibit non-NMDA-induced responses, depending on the zinc concentration (Mayer *et al.*, 1988; Rassendren *et al.*, 1990).

In contrast to the various effects exerted by numerous cations, anions have not featured prominently as modulators of EAA receptors, which is not surprising since these ions are unlikely to interfere with essentially cation-permeable ligand-gated ion channels. A notable exception to this general exclusion is the chaotropic anion, thiocyanate (SCN^-). In the presence of SCN^-, binding of the non-NMDA agonist, AMPA to brain membranes was considerably enhanced, but the binding of KA remained unaffected (Honore & Nielsen, 1985; Murphy *et al.*, 1987). This useful finding resulted in the majority of radioligand binding assays for AMPA employing thiocyanate ions as an experimental tool to increase the affinity of the receptor for this agonist (Honore & Drejer, 1988), allowing differentiation between AMPA and KA binding. The mechanism of action for SCN^- to enhance AMPA binding is unknown and perhaps more important, the functional effects of SCN^- ions on non-NMDA receptors have rarely been addressed.

In the present study we investigated the functional effects

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of SCN^- on expressed non-NMDA receptors in rat brain mRNA-injected *Xenopus* oocytes, and also on native non-NMDA receptors maintained in cultured cerebellar neurones. In particular we examine whether an anion-selective binding site exists on the non-NMDA receptor complex which is capable of selectively modulating receptor function following activation by AMPA or KA.

A preliminary report of part of this work has appeared previously (Bowie & Smart, 1991).

Methods

Extraction and injection of Xenopus laevis *oocytes*

Female *Xenopus laevis* toads (140–200 g) were anaesthetized in a cold solution of ethyl-m-aminobenzoate (Tricaine) prior to the removal of part of an ovary containing 50–100 oocytes. Oocytes were manually separated from the ovary wall and placed in modified Barth's medium (MBM) containing (mM) at pH 7.6: NaCl 110, KCl 1, $NaHCO_3$ 2.4, Tris HCl 7.5, $Ca(NO_3)_2$ 0.33, $CaCl_2$ 0.41, $MgSO_4$ 0.82 and streptomycin, 10 μg ml^{-1}. Only oocytes at developmental stages V and VI (Dumont, 1972) were selected for mRNA injection. Each oocyte was injected into the equatorial region with 50 nl of a 1 mg ml^{-1} mRNA solution. Injected oocytes were then incubated at 18°C for 2–3 days to allow the expression of receptor protein. After this period, all oocytes were stored in MBM at 10°C and re-fed with fresh MBM every 2 days. This prolonged the survival of the oocytes for up to 4–5 weeks. Translationally-active mRNA was extracted from 3–6 week-old rat brain (without the cerebellum) by a guanidinium thiocyanate/caesium chloride method and purified by oligo-dT column chromatography (Chirgwin *et al.*, 1976).

Dissociated cultures of cerebellar neurones

Cerebellar cultures were prepared as described previously (Smart, 1992). Briefly, cerebellar cortices were removed from embryonic day 17–18 rats (E17–18) and chopped into slices in ice-cooled plating medium containing: minimum essential medium (MEM) with 10% v/v foetal calf serum, 10% v/v horse serum, 2 mM glutamine, 0.6% w/v glucose and 100 units ml^{-1} penicillin-G and 100 μg ml^{-1} streptomycin. The neurones were mechanically dissociated through nylon mesh (diameter 210 μm) and plated onto poly-L-lysine coated dishes and incubated at 37°C with 95% air/5% CO_2. After 4 days the plating medium was removed and replaced with a growth medium which retained the contents of the plating medium except for the foetal calf serum. Large cerebellar neurones were used for electrophysiology after 20 days in culture and were distinguished from granule cells on the basis of soma diameter and dendritic morphology (see Smart, 1992 for discussion).

Electrophysiology
Intracellular two-electrode voltage clamp in Xenopus *oocytes*

Xenopus oocytes were secured to the base of a Perspex bath (volume 0.5–1 ml) coated with Sylgard following placement within a circle of insect pins. Cells were impaled with two microelectrodes manufactured from thin-walled borosilicate glass and filled with 3 M KCl solution providing resistances of 5–10 MΩ for the voltage and 1–2 MΩ for the current microelectrodes. Electrophysiological recordings were made using an Axoclamp 2A amplifier in two-electrode voltage clamp mode and membrane currents were monitored on a Brush-Gould thermal chart recorder (2200). All drugs were applied in frog Ringer via the bath superfusion system (rate 8–10 ml min^{-1}) through a gravity fed inlet placed 5 mm away from the oocyte surface. The frog Ringer contained (mM): NaCl 110, KCl 2, HEPES 5, $CaCl_2$ 1.8, pH 7.4. Only oocytes with input resistances of 1–5 MΩ and resting membrane potentials of −40 to −60 mV were accepted for experimentation.

Whole-cell recording from cultured neurones

Experiments were performed with a List EPC7 amplifier by the whole-cell patch clamp recording method. Patch electrodes were fabricated from thin walled borosilicate glass and filled with a pipette solution containing (mM): KCl 150, $CaCl_2$ 0.279, $MgCl_2$ 1, Na-EGTA 0.5, HEPES 10, pH 7.1. Pipettes had resistances ranging from 1–50 MΩ. The neurones were viewed under phase-contrast optics and continually superfused in a 35 mm culture dish (volume 1 ml) with a Krebs solution containing (mM): NaCl 140, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2, glucose 11, HEPES 5, pH 7.4 at 30°C. Drugs and Krebs solutions were applied to the neurones by a rapid perfusion system consisting of a multibarrelled pipette made from Quad glass (Q-tube, Clarks Electromedical). The concentration-response relation for AMPA was determined with a U-tube perfusion system for drug application (Krishtal & Pidoplichko, 1980; Fenwick *et al.*, 1982). The U- or Q-tubes were placed within 300 μm of the cell under study.

Results

Differential antagonism of non-NMDA induced responses by thiocyanate ions

In *Xenopus* oocytes injected with rat brain mRNA and voltage clamped at −60 mV holding potential, bath-application of AMPA (4–100 μM) or willardiine (10–320 μM) produced small reproducible inward membrane currents (11 ± 1.4 nA for AMPA, $n = 10$; 10 ± 2 nA for willardiine, $n = 6$) and associated conductance increases when compared to the larger responses induced by 80 μM KA in the same cells (84 ± 8 nA; $n = 9$). The responses evoked by higher concentrations of AMPA (50–100 μM) also differed by exhibiting an apparent desensitization during continued application of the agonist. This contrasted with the apparently maintained amplitudes of the KA- or willardiine-induced currents and with responses induced by low concentrations of AMPA (4–10 μM; Figure 1). Desensitizing AMPA-induced responses were characterized by a discernible peak inward current which decayed to a steady-state during agonist application. Following the removal of AMPA, the membrane current quite often exhibited a rebound inward tail current, prior to decaying back to the holding current (Figure 1a).

The inclusion of 50 μM–4 mM thiocyanate ions (SCN^-; sodium salt) in the Ringer solution did not affect the resting membrane potential or input conductance of mRNA-injected or uninjected *Xenopus* oocytes. However, responses induced by AMPA (4–100 μM) were selectively antagonized by SCN^- with the peak and steady-state currents for 100 μM AMPA reduced by 55 ± 4 and 74 ± 3% respectively ($n = 8$). The onset of antagonism and the recovery from inhibition was rapid and showed no evidence of any use-dependence. In contrast to AMPA-induced responses, 2 mM SCN^- did not significantly affect the amplitude of either 80 μM KA-induced (102 ± 5%) or 100 μM willardiine-induced (99 ± 3%) membrane currents ($n = 5$; Figure 1b/c).

Thiocyanate ions non-competitively inhibited AMPA-induced currents

The mechanism of inhibition of AMPA-induced currents by SCN^- was analyzed using concentration-response curves obtained by measuring the steady-state current amplitudes of the responses to AMPA (2.5–200 μM). Bath-application of SCN^- (2 mM) inhibited the AMPA concentration-response curve in a non-competitive manner, simply depressing the

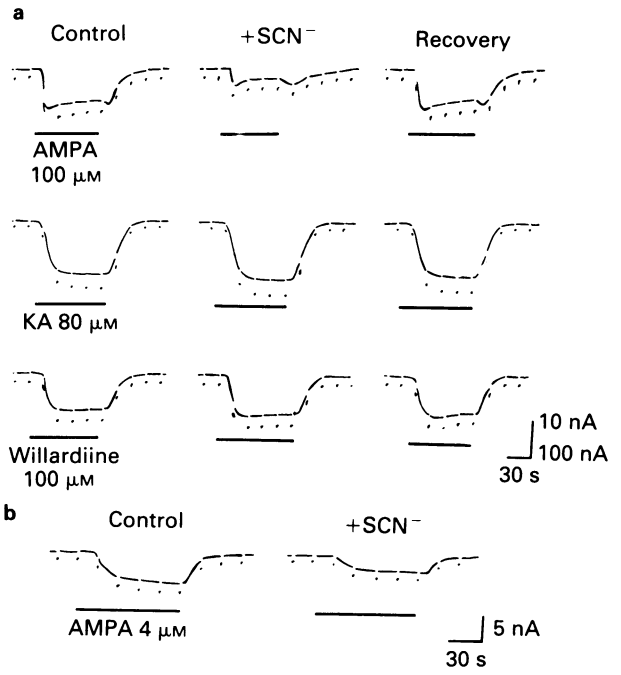

Figure 1 Thiocyanate ions selectively inhibited α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced responses. (a) Inward membrane currents were induced by AMPA (100 μM), kainate (KA, 80 μM) or willardiine 100 μM in rat brain mRNA-injected oocytes voltage clamped at a holding potential of − 60 mV in the presence and absence of 2 mM SCN^-. In this and some of the subsequent figures, hyperpolarizing voltage commands (5–10 mV, 1.5 s, 0.05 Hz) were applied to monitor the membrane conductance and the agonists were applied for the duration indicated by the solid lines. Note the decaying responses to AMPA and the lower current amplitudes to AMPA and willardiine compared to KA. The 10 nA calibration only applies to currents induced by AMPA and willardiine. (b) Low concentrations of AMPA exhibited maintained current amplitudes in the presence or absence of 2 mM SCN^-.

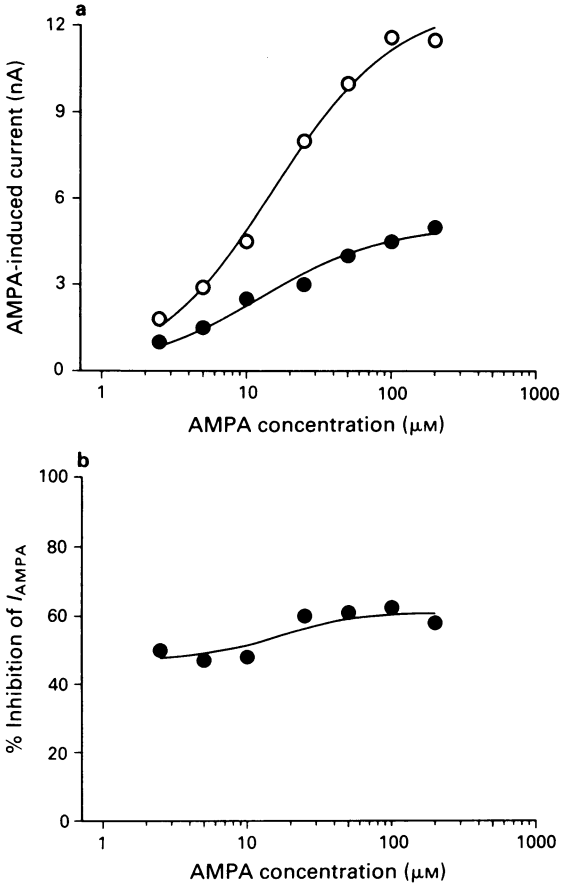

Figure 2 Thiocyanate ions non-competitively antagonize α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced responses. (a) Typical concentration-response curves for responses to AMPA were obtained in the presence (●) and absence (○) of 2 mM SCN^-. The curves were fitted using a receptor model state function of the form, $Y/Y_{max} = \{[A]/[A] + K_A\}^n$, where A, K_A and n represent agonist concentration, dissociation constant and the Hill coefficient. The data were fitted according to a Marquadt non-linear least squares routine. K_A and n were estimated in control Ringer to be 12.7 ± 1.2 μM and 1.2 ± 0.33 ($r = 0.9968$); and in 2 mM SCN^-, 12.5 ± 1.9 μM and 1.0 ± 0.5 ($r = 0.9902$). (b) The percentage inhibition of the AMPA-induced responses by 2 mM SCN^- are plotted against the AMPA concentration. Data were calculated from (a).

curve without inducing any lateral shifting (Figure 2a). Estimation of the EC_{50}s for AMPA in the absence and presence of SCN^- were similar at 19 and 15 μM respectively. The corresponding percentage-inhibition plot for AMPA-induced responses revealed that the antagonism produced by SCN^- was only slightly dependent on the agonist concentration, with 4 μM AMPA-induced responses inhibited by 50 ± 4% and responses to 100 μM AMPA reduced by 63 ± 3% ($n = 8$; Figure 2b). The absence of any noticeable shift by an antagonist in a dose-response curve is often formally interpreted as indicating no change in the apparent affinity of the receptor for an agonist. This may be true for the action of SCN^- on the non-NMDA receptors; however, we cannot discount an effect of SCN^- on other processes preceding ion channel activation.

In Figure 3a, the membrane current induced by AMPA in control Ringer solution displayed a slow decline from the peak response, but did not reveal any evidence of a rebound tail current following removal of the agonist (cf. Figure 1a). In the presence of low concentrations of SCN^- (0.5 mM), the AMPA-induced response was depressed and the slow decline in the current amplitude was also without any rebound tail current (Figure 3a); however, after exposure of the oocyte to higher concentrations of SCN^- (> 1 mM), the AMPA-induced responses were antagonized to a greater extent and following cessation of AMPA application an inward rebound tail current was often revealed. The concentration of SCN^- required to produce a half-maximal inhibition (IC_{50}) in the steady-state AMPA-induced response was determined from an inhibition plot constructed for AMPA responses in the presence of 50 μM–4 mM SCN^-. In Figure 3b, the response induced by 50 μM AMPA was reduced in a concentration-dependent manner by SCN^- with an IC_{50} of 1 mM.

Thiocyanate ions and desensitization of non-NMDA receptors

The decline in the AMPA-induced peak current was observed on rapidly superfusing the oocyte at 8–10 ml min^{-1} and could represent a component of receptor desensitization since it was also accompanied by a decline in the membrane conductance. This fade in the peak current was assessed by calculating a ratio of the steady-state current (I_{ss}), measured after 90 s in AMPA-containing Ringer, to the initial peak current (I_p). A clear peak current which exhibited desensitization to a smaller steady-state response was only observed at AMPA concentrations > 50 μM and the corresponding ration, I_{ss}/I_p, was reduced with increasing AMPA concentration (Figure 4b). This reduction in the I_{ss}/I_p ratio was accounted for by a reduced I_{ss}, suggesting that the measurement of I_{ss} was more susceptible to the effect of desensitization than I_p. For example, rapid bath-application of 200 μM AMPA ($I_{ss}/I_p = 0.67 \pm 0.04$, $n = 4$) induced a current which desensitized more so than a response induced by 100 μM AMPA (0.83 ± 0.03, $n = 10$; Figure 4a). Declining AMPA-

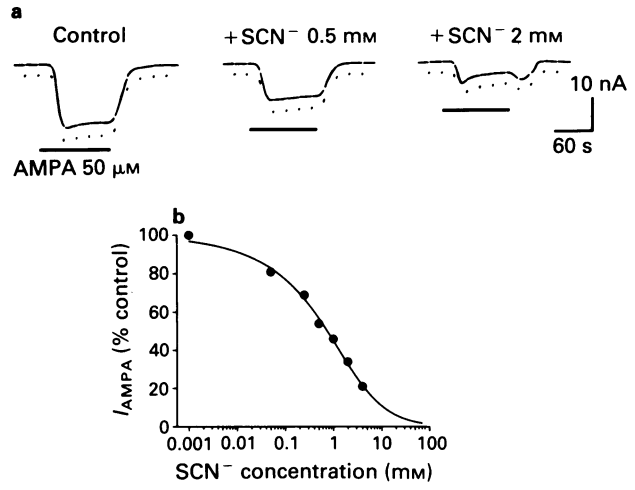

Figure 3 Dependence of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced response antagonism on SCN^- concentration. (a) Inward currents induced by AMPA (50 μM) were recorded from a single oocyte voltage clamped at − 60 mV holding potential in control Ringer and 0.5 or 2 mM SCN^-. Note the initial decay of the AMPA-induced response and the appearance of an inward rebound tail current in the presence of 2 mM SCN^- following the removal of AMPA. (b) Dose-response analysis for the inhibition by 1 μM–4 mM SCN^- of a response to AMPA (50 μM). The data were fitted to the equation, $Y'/Y = 1-\{[B]/([B] + K_B)\}$, whereby Y' represents the AMPA-induced current response in the presence of SCN^- and Y the control response amplitude. B and K_B represent SCN^- concentration and the apparent dissociation constant respectively. K_B was estimated as 3.1 ± 0.56 mM and n = 0.43 ± 0.04.

induced currents were generally not observed if a slower agonist-perfusion rate (< 5 ml min^{-1}) was employed (Bowie, 1991).

The rebound inward tail current amplitude was also more clearly resolved in responses to the higher AMPA concentrations (50–200 μM; Figure 4a) and could always be correlated with the onset of desensitization in the peak AMPA-induced current.

By using AMPA concentrations that induced a desensitizing current response (> 50 μM), the peak AMPA-induced current was inhibited to a lesser extent than the steady-state current in the presence of 2 mM SCN^-. Accordingly, I_{ss}/I_p for 100 μM AMPA-induced currents was reduced by SCN^- from 0.86 ± 0.06 to 0.52 ± 0.06 ($n = 8$; Figure 4b).

The reduction in the I_{ss}/I_p ratio following treatment with SCN^-, raised the possibility that SCN^- might be inhibiting AMPA-induced responses by increasing receptor desensitization. This was investigated using the plant lectin, concanavalin-A (Con-A), which reduces desensitization of glutamate or AMPA-induced responses mediated by neuronal non-NMDA receptors (Mayer & Vyklicky, 1989). Pretreatment of mRNA-injected oocytes for 30–45 min with 10 μM Con-A selectively enhanced the responses elicited by 100 μM AMPA when compared to 80 μM KA-induced responses. Both I_p and I_{ss} for the AMPA responses were enhanced by 25 ± 3% and 40 ± 5% respectively over the control values prior to Con-A treatment ($n = 3$). This differential effect of Con-A resulted in only a small increase in the I_{ss}/I_p ratio for AMPA-induced (100 μM) responses from 0.83 ± 0.03 ($n = 10$) to 0.9 ± 0.04 ($n = 3$; Figure 4b). This enhancement was apparently irreversible and could still be observed up to 60 min following exposure to Con-A.

In the presence of 2 mM SCN^-, the residual I_p and I_{ss} responses to 100 μM AMPA in Con-A-treated oocytes were reduced by 54 ± 2% and 67 ± 7% respectively (Figure 4c). In comparison, prior to the treatment with Con-A in the same mRNA-injected oocytes, SCN^- reduced I_p and I_{ss} by 55 ± 4% and 74 ± 3% ($n = 8$). Lower concentrations of Con-A

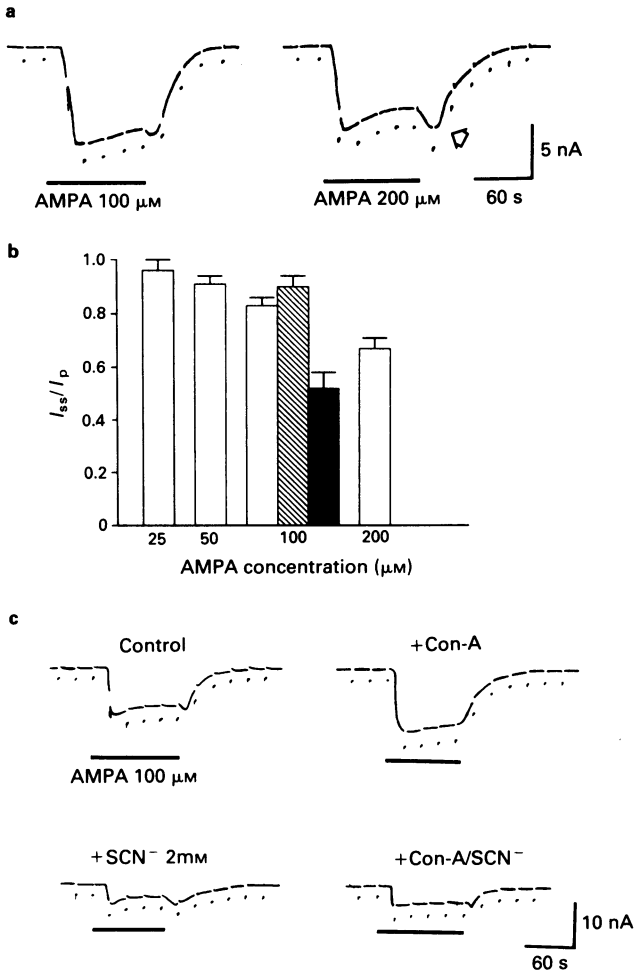

Figure 4 Desensitization of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-induced responses. Effect of concanavalin A (Con-A) and SCN^-. (a) Saturating concentrations of AMPA (100–200 μM) evoked desensitizing responses with clear peak inward currents and rebound tail currents which appeared after the removal of AMPA. The tail currents (arrow) were more clearly resolved with higher concentrations of AMPA which displayed more overt desensitization. (b) A ratio of the steady-state current (I_{ss}), measured after 90 s in the presence of AMPA, to the initial peak inward current (I_p), is plotted against the agonist concentration (open columns). Increasing AMPA concentration caused this ratio to decrease, consistent with dose-dependent desensitization. For oocytes exposed to 100 μM AMPA, pretreatment with 10 μM Con-A increased the I_{ss}/I_p ratio (hatched column), whereas in other oocytes, addition of 2 mM SCN^- reduced this ratio (solid column). All values represent means ± s.e.mean from 3–10 mRNA-injected oocytes. (c) Responses evoked by 100 μM AMPA in a single oocyte voltage clamped at − 60 mV were recorded in four different solutions including: control Ringer; + 2 mM SCN^-; and following a recovery from SCN^- (not shown), after a 30 min exposure to 10 μM Con-A; and finally after Con-A treatment, in 2 mM SCN^-. The AMPA-induced responses in control Ringer exhibited a rebound tail current which was lost in Con-A-treated oocytes.

(1 μM) were less effective in reducing the level of non-NMDA receptor desensitization.

Effect of thiocyanate ions on the antagonism of kainate-induced responses by AMPA

Many studies on native non-NMDA receptors on catfish retinal neurones (O'Dell & Christensen, 1989), and mammalian hippocampal and spinal cord neurones (Zorumski & Yang, 1988; Thio *et al.*, 1991; Patneau & Mayer, 1991; see Gasic & Hollman, 1992, for review) and also on expressed

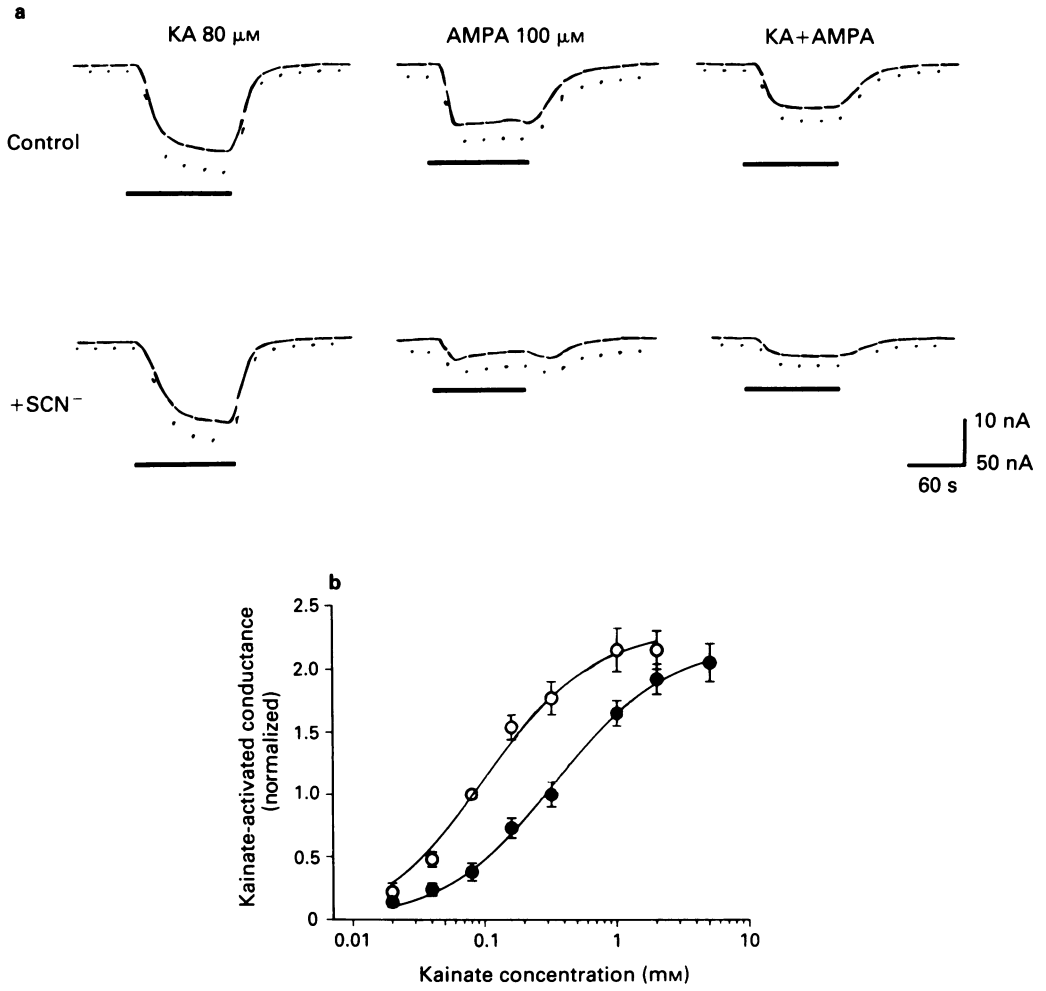

Figure 5 Antagonism by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) of responses to kainate (KA) are unaffected by SCN^-. (a) Sample responses of rat brain mRNA-injected oocytes to bath-applied KA (80 μM), AMPA (100 μM) and combinations of 80 μM KA + 100 μM AMPA in the absence and presence of 2 mM SCN^-. Note the smaller amplitude currents induced by AMPA compared to responses evoked by KA. The 10 nA calibration applies only to the AMPA-induced responses. Holding potential − 60 mV. (b) Concentration-response curves for responses to KA in the absence (○) and presence (●) of 100 μM AMPA were fitted with the state function and method described in Figure 2. All values are mean ± s.e.mean and normalized to the response amplitude of 80 μM KA in control Ringer. For control oocytes, the apparent K_A was estimated at 67.6 ± 7 μM and n = 1.4 ± 0.2. In the presence of 100 μM AMPA, K_A = 302.2 ± 17.8 μM and n = 1.1 ± 0.17 (n = 3).

non-NMDA receptors in *Xenopus* oocytes (Verdoorn & Dingledine, 1988), have observed that responses to KA can be inhibited by co-application of either quisqualate or AMPA. These studies have proposed that both AMPA and KA may be acting on the same receptor complexes, since the inhibition of KA responses is of a competitive nature. This conclusion received some support from more recent molecular cloning studies which have demonstrated that particular non-NMDA receptor subunits derived from cDNAs are sensitive to both AMPA and KA (e.g. GluR1-4; although some are relatively insensitive to AMPA, e.g., GluR6; see Gasic & Hollman, 1992, for review). In this study, using rat brain mRNA-injected oocytes, near saturating concentrations of AMPA (100 μM) consistently inhibited the response induced by 80 μm KA by 54 ± 2% (n = 10; Figure 5a). The antagonism was rapid in onset and the recovery was not apparently use-dependent. The KA concentration-response curve was antagonized by AMPA (100 μM) producing a lateral shift in the curve, suggesting a competitive mode of antagonism (Figure 5b). The apparent dissociation constants (K_A) for KA, estimated from a two-independent binding site receptor model (Bowie, 1991) applied to the data in the absence and presence of 100 μM AMPA, were calculated as, 67.6 ± 7 μM and 302.2 ± 17.8 μM (n = 3) respectively.

In the presence of SCN^- (2 mM), the KA-induced response was unaffected, but the response to AMPA was reduced by 74 ± 3%; furthermore, AMPA was now more effective in inhibiting the KA response (82 ± 5%; n = 3; Figure 5a). This reduction in the response amplitude to KA plus AMPA in the presence of SCN^- could be mostly accounted for by the SCN^--induced reduction in the AMPA response alone. Thus AMPA may still be equi-effective as an apparent antagonist of responses to KA even in the presence of SCN^-. However, in receptor expression studies, we cannot of course discount the possibility that SCN^- may have a selective action on particular non-NMDA receptors expressed from heterogeneous mRNA injected into *Xenopus* oocytes; but, it should be noted, that such a situation also pertains to neuronal studies, which are likely to contain heterogeneous populations of non-NMDA receptors.

AMPA-induced responses on cultured cerebellar neurones are inhibited by thiocyanate ions

Large cultured cerebellar neurones were whole-cell voltage clamped at − 60 mV holding potential and AMPA (10 μM) was rapidly applied from an adjacent four-barrelled flow pipette (Q-tube). The membrane currents induced by AMPA

displayed a characteristic large peak current which rapidly decayed to a much smaller steady-state current (Figure 6a). Application of 2 mM SCN^- via the rapid perfusion pipette did not alter the holding current nor the resting conductance of these neurones. On co-application of SCN^- with AMPA, the peak current responses were slightly reduced by 22 ± 5%, but the steady-state responses were more susceptible to antagonism by SCN^-, being reduced by 45 ± 7% ($n = 4$; Figure 6a). To reproduce the responses observed with expressed non-NMDA receptors in *Xenopus* oocytes, the flow rate from the Q-tube was reduced such that the fast peak current to AMPA was not resolved and only the steady-state current remained (Figure 6a). Application of 2 mM SCN^- reduced the steady-state current by 41% and revealed a fast decaying inward peak current in response to 10 μM AMPA. This reduction in the AMPA response by SCN^- was not use-dependent and a comparable level of inhibition could be achieved by co-application of 10 μM AMPA and 2 mM SCN^- without any prior incubation in SCN^--containing Krebs solution.

The type of antagonism exerted by SCN^- on the AMPA-induced responses was assessed from the equilibrium concentration-response curve characteristics. Different concentrations of AMPA were rapidly applied from a U-tube (see Methods) in the absence and presence of SCN^- and the steady-state inward current was measured as a more reliable indicator of agonist efficacy compared to the peak current (Figure 6c). A two-independent binding site receptor model was used to determine the apparent dissociation constant (K_A) for AMPA. In control Krebs solution, K_A was 6.91 ± 0.35 μM ($n = 3$) remaining unaffected in 2 mM SCN^- where K_A was estimated as 7.02 ± 0.4 μM. Similar to the dose-response curve for expressed non-NMDA receptors in oocytes, SCN^- depressed the AMPA dose-response curve in cerebellar neurones in a non-competitive manner with little dependence on the agonist concentration.

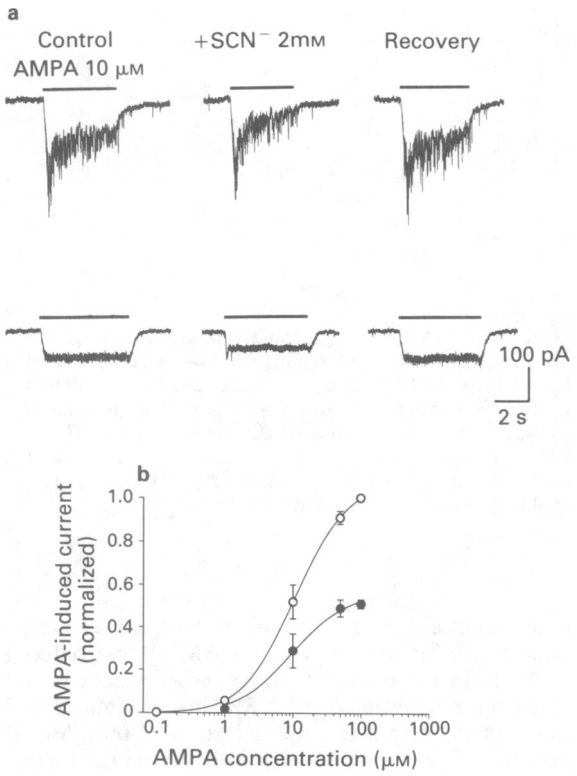

Figure 6 Responses to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) on cultured cerebellar neurones are antagonised by SCN^-. (a) Upper traces, in a large cultured cerebellar neurone voltage clamped at − 60 mV holding potential, rapid-application of 10 μM AMPA in control Krebs from a Q-tube evoked a rapidly decaying current with a steady-state component. SCN^- (2 mM) was also applied from the Q-tube, note the greater reduction in the steady-state current compared to the peak current. Lower traces, by slowing the rate of drug application (10 μM AMPA) the fast peak current was not resolved leaving a predominantly steady-state AMPA-induced current. SCN^- reduced the amplitude of the AMPA current revealing a small initial inward tail current. (b) Concentration-response analysis for responses to AMPA in the absence (○) and presence (●) of 2 mM SCN^-. Values are means ± s.e.mean from 5 neurones. The data were fitted with the state function described in Figure 2, providing apparent values of $K_A = 6.91 \pm 0.35$ μM and $n = 1.43 \pm 0.05$ in control Krebs, and $K_A = 7.02 \pm 0.4$ μM and $n = 1.33 \pm 0.13$ in SCN^-. Different concentrations of AMPA were delivered from a U-tube in control Krebs or in 2 mM SCN^-.

Discussion

SCN ions differentially modulate AMPA binding and AMPA-induced responses

Thiocyanate ions belong to a group of agents known as chaotropic ions which favour the transfer of apolar groups into the water phase (Hatefi & Hanstein, 1969). Traditionally chaotropic ions have been used as general biochemical reagents to dissociate a variety of particulate proteins and multicomponent enzymes (Sawyer & Puckridge, 1973). In radioligand binding studies of non-NMDA receptors, chaotropic ions increased the specific binding of [^{3}H]-AMPA in rat brain membranes by up to 800% of basal binding at concentrations ranging from 1–100 mM (Honore & Nielsen, 1985; Murphy *et al.*, 1987; Olsen *et al.*, 1987; Honore & Drejer, 1988; Nielsen *et al.*, 1988; Shahi & Baudry, 1992). Honore & Drejer (1988) have proposed that AMPA can bind to a receptor which exists in two interconvertible states of low and high affinity with SCN^- favouring formation of the high affinity state. Interestingly, the binding of other non-NMDA agonists, such as KA, are unaffected by the presence of SCN^- (Murphy *et al.*, 1987; Honore & Nielsen, 1988).

This selective action of SCN^- was also observed in our electrophysiological analyses on non-NMDA receptors expressed in *Xenopus* oocytes, where perhaps surprisingly, SCN^- inhibited responses to AMPA without affecting KA-induced responses. The concentrations of SCN^- used in the present study (2–10 mM) were lower than those routinely employed in binding studies (typically 100 mM); however, even using comparable concentrations of SCN^- in binding studies, [^{3}H]-AMPA binding was still clearly enhanced (Shahi & Baudry, 1992). The AMPA concentration-response curve for expressed non-NMDA receptors was antagonized in a non-competitive manner by SCN^-, suggesting that SCN^- is unlikely to be competing for the agonist recognition site. This antagonism is probably not due to an expression artifact of the *Xenopus laevis in vitro* translation system, since a similar mode of antagonism was observed when SCN^- was studied on native non-NMDA receptors in cultured cerebellar neurones.

Mechanism of antagonism of AMPA-induced responses by SCN^-

The antagonism of AMPA-induced responses by SCN^- could occur by a variety of mechanisms, including: (i) ion channel block, (ii) enhancing receptor desensitization, (iii) allosteric modulation of ion channel opening by specific agonists, (iv) chelation/inactivation of the agonist in solution.

Non-NMDA agonist-gated ion channels are permeable only to cations and on this basis alone, it appears very unlikely that SCN^- could exert a blockage of the open channel by entering the channel lumen. Moreover, for both expressed receptors in *Xenopus* oocytes and native receptors in cerebellar neurones, the degree of block by SCN^- was only minimally dependent on the agonist concentration, in-

dicating that the blockade is not selective for just the open states of the ion channel.

By enhancing desensitization, SCN^- could conceivably effect a non-competitive depression of the AMPA dose-response curve. At high AMPA concentrations (> 100 μM), where apparent desensitization of the response was maximal, SCN^- antagonized the steady-state AMPA response more than the peak current, thereby decreasing the I_{ss}/I_p ratio. In previous studies on hippocampal neurones, the rapidly desensitizing AMPA-induced current suggests that I_{ss} represents the AMPA response at a greater level of desensitization since it was markedly enhanced by Con-A treatment, whereas I_p was relatively insensitive (Mayer & Vyklicky, 1989). In addition, a study of the single channel currents activated by quisqualate on hippocampal neurones, revealed that I_p may be carried by rapidly-inactivating high conductance channels (single channel conductance (γ) = 35pS; Tang *et al.*, 1989), whereas the maintained current (approximating to I_{ss}) was carried mostly by low conductance channels (γ = 8pS; Jahr & Stevens, 1987; Cull-Candy & Usowicz, 1987; Ascher & Nowak, 1988).

In our study, the rapidly-inactivating membrane current evoked by AMPA was clearly not fully resolved due to the inherent limitations associated with rapid drug application onto large cells such as the *Xenopus* oocyte with a cell diameter of approximately 1 mm. Consequently, I_p does not represent AMPA activating a non-NMDA receptor(s) at a minimal level of desensitization and it was therefore not possible to determine absolutely whether SCN^- ions selectively affected I_{ss} or I_p. However, using cultured cerebellar neurones, we resolved both the rapidly densensitizing and steady-state currents induced by AMPA, and SCN^- appeared to antagonize the steady-state responses to a greater extent. The apparently greater effect of SCN^- on I_{ss} might suggest that SCN^- is enhancing desensitization perhaps by causing the channel to adopt a low conductance open state conformation (cf. Jahr & Stevens, 1987; Cull-Candy & Usowicz, 1987; Ascher & Nowak, 1988).

One feature of the present results which is apparently inconsistent with this model was the observation that Con-A treatment, which reduces receptor desensitization, did not affect the antagonism of AMPA-induced responses by SCN^-. We cannot discount the possibility that SCN^- may enhance desensitization by a Con-A insensitive mechanism. This possibility is supported by the antagonism of KA-induced responses by AMPA where both agonists are thought to bind to the same receptor protein(s). The degree of antagonism was also suggested to be due to the level of desensitization induced by AMPA (Kiskin *et al.*, 1986; Patneau & Mayer, 1991); this interaction was insensitive to Con-A treatment (Mayer & Vyklicky, 1989) and also in our study, to SCN^- ions.

If SCN^- can modulate receptor desensitization, then this might explain the increased binding of [^{3}H]-AMPA by SCN^- and also the reduction in the AMPA-induced membrane current. The nature of radioligand binding studies necessarily requires an incubation of the agonist/antagonist with the receptor preparation for longer periods of time, compared to that routinely used in electrophysiological studies. This suggests that the non-NMDA receptors in binding studies would have entered into one or more desensitized states which is usually associated with high affinity binding sites for ligands. We would then predict that the response amplitude and the EC_{50} for AMPA would both be reduced by SCN^- (cf. Patneau & Mayer, 1990; Barnard & Henley, 1990); however, our data clearly show that the EC_{50} is apparently unaffected.

AMPA-induced rebound tail currents

The rebound tail current observed following washout of AMPA was enhanced in the presence of SCN^-. This phenomenon also appeared to be related to receptor desensitization, but in other studies, desensitization of AMPA responses have been clearly demonstrated to occur either in the absence (Kiskin *et al.*, 1986; Trussell *et al.*, 1988; Zorumski & Yang, 1988; Mayer & Vyklicky, 1989; O'Dell & Christensen, 1989), or presence of a rebound current (Vyklicky *et al.*, 1986; Vlachova *et al.*, 1987; Tang *et al.*, 1988; Perouansky & Grantyn, 1989). One explanation for the production of rebound tail currents involves a 'self-block' of the ion channel by the agonist; a concept originally proposed for nicotinic agonists at the frog neuromuscular junction (Adams, 1975). Relief of the block following washout of the agonist might allow re-activation of the receptor by previously 'trapped' agonist molecules resulting in a small tail current. A similar mechanism was also suggested to account for the 'after-current' following the discontinuation of quisqualate application to spinal neurones (Vyklicky *et al.*, 1986; Vlachova *et al.*, 1987). However, the structure of the AMPA molecule with two, presumably dissociated carboxyl groups, suggests that the penetration of AMPA molecules into the cation-selective ion channel would probably be thermodynamically unfavourable. Moreover, a similar rebound current induced by AMPA on expressed non-NMDA receptors in *Xenopus* oocytes, was devoid of any voltage sensitivity (Geoffroy *et al.*, 1991). The rebound current was accounted for by postulating that a decrease in receptor occupancy during washout of the agonist allowed a fast re-activation of the receptor, producing an increase in the membrane current (Geoffroy *et al.*, 1991). The manifestation of the AMPA-induced 'rebound' current in SCN^- containing Ringer solution, may reflect a dissociative effect of SCN^- on non-NMDA receptor protein structure, by binding to a discrete site from the agonist recognition site. The possibility of SCN^- ions allosterically modulating channel gating and possibly also enhancing desensitization, has not yet been discounted.

In the presence of SCN^-, the shape of the AMPA dose-response curve, exhibiting a reduced maximum and with no lateral shift, suggests that SCN^- is not chelating or chemically inactivating the agonist molecules (Smart & Constanti, 1982). A recent study by Shahi & Baudry (1992) in hippocampal slices monitored the extracellular excitatory post-synaptic potential (e.p.s.p.). Ionophoresis of SCN^- ions resulted in an enhanced slope to the e.p.s.p. by approximately 25%. This was an unexpected result based on our findings of SCN^- inhibiting non-NMDA receptors. Molecular cloning studies have now revealed a number of different non-NMDA receptor cDNAs (see Gasic & Hollman, 1992) which may have discrete distributions in the CNS and different functional properties (Keinanen *et al.*, 1990; Sommer *et al.*, 1990; Lambolez *et al.*, 1992; Gasic & Hollman, 1992). Therefore, it is possible that various heteromeric combinations of these subunits may be differentially sensitive to SCN^- ions.

Discrete binding sites for non-NMDA agonists on the same receptor complex?

In oocytes injected with rat brain mRNA, the membrane currents evoked by KA and AMPA are thought to be mediated by common receptor complexes (Verdoorn & Dingledine, 1988). This also concurs with the notion of a common binding site for AMPA and KA on some native or expressed non-NMDA receptors (Boulter *et al.*, 1990; Keinanen *et al.*, 1990; Patneau & Mayer, 1991). Patneau & Mayer (1991) proposed that both agonists bind to the same receptor and show mutual competition for the agonist recognition site. For the non-NMDA receptors expressed in our study, discrete binding sites for AMPA and KA is also a possibility, even on the same receptor protein(s), since SCN^- ions selectively antagonized responses to AMPA compared to KA-induced responses. However, expression of non-NMDA receptors in *Xenopus* oocytes from mRNA may yield heterogeneous populations of non-NMDA receptors. Two alternative hypotheses are now possible to explain the effects of SCN^-: AMPA and kainate may bind to completely different

receptor populations and SCN^- will bind only to the 'AMPA-sensitive' receptors. This would be compatible with our results of SCN^- selectively inhibiting responses to AMPA, but this concept would not easily explain the competitive shift in the kainate dose-response curve induced by AMPA, nor the extra depression of responses to kainate caused by AMPA in the presence of SCN^-; alternatively, there might be a mixture of AMPA/kainate-sensitive receptors and discrete populations of AMPA and kainate-sensitive receptors. Presumably in this scenario, SCN^- will bind to more than one population of receptors. This is a complex situation and the behaviour of the system cannot be easily predicted without constraining assumptions for which, at present, we do not have any experimental justification.

The most parsimonious conclusion that can be drawn from our results is that the non-competitive antagonism produced by SCN^-, suggests that this ion does not displace AMPA from the binding site but probably modulates channel gating from a novel allosteric site. Furthermore, as an antagonist in this study, SCN^- has the novel distinction for non-NMDA receptors, of being able to differentiate between AMPA and KA-induced responses.

This work was supported by the MRC, Wellcome Trust and SERC. We are grateful to Mike Webb (Sandoz) for the provision of rat mRNA. We thank our colleagues at The School of Pharmacy for helpful discussions.

References

ADAMS, P.R. (1975). A study of desensitization using voltage clamp. *Pflügers Arch.*, **360**, 135–144.

ASCHER, P. & NOWAK, L. (1988). Quisqualate and kainate-activated channels in mouse central neurones in culture. *J. Physiol.*, **399**, 227–245.

BARNARD, E.A. & HENLEY, J.M. (1990). The non-NMDA receptors: types, protein structure and molecular biology. *Trends Pharmacol. Sci.*, **11**, 500–507.

BOULTER, J., HOLLMANN, M., O'SHEA-GREENFIELD, A., HARTLEY, M., DENERIS, E., MARON, C. & HEINEMANN, S. (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. *Science*, **249**, 1033–1037.

BOWIE, D. (1991). Functional properties of vertebrate non-NMDA excitatory amino acid receptors expressed in *Xenopus laevis* oocytes. *Ph.D. Thesis, London University.*

BOWIE, D. & SMART, T.G. (1991). Non-NMDA receptors expressed in *Xenopus laevis* oocytes after injection with rat brain mRNA are differentially antagonised by thiocyanate ions. *J. Physiol.*, **434**, 95P.

CHIRGWIN, J.M., PRYZBYLA, A.E., MACDONALD, R.J. & RUTTER, W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, **18**, 5249–5299.

CHRISTENSEN, B.N. & HIDA, E. (1990). Protonation of histidine groups inhibits gating of the quisqualate/kainate channel protein in isolated catfish cone horizontal cells. *Neuron*, **5**, 471–478.

CULL-CANDY, S.G. & USOWICZ, M.M. (1987). Multiple-conductance channels activated by excitatory amino acids in cerebellar neurones. *Nature*, **325**, 525–528.

DUMONT, J.N. (1972). Oogenesis in *Xenopus laevis* (Daudin), 1. Stages of oocyte development in laboratory maintained animals. *J. Morphol.*, **136**, 153–180.

ENOMOTO, R., OGITA, K., HAN, D. & YONEDA, Y. (1992). Differential modulation by divalent cations of [^{3}H]MK-801 binding in brain synaptic membranes. *J. Neurochem.*, **59**, 473–481.

FENWICK, E.M., MARTY, A. & NEHER, E. (1992). A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol.*, **331**, 577–597.

FORSYTHE, I.D., WESTBROOK, G.L. & MAYER, M.L. (1988). Modulation of excitatory synaptic transmission by glycine and zinc in cultures of mouse hippocampal neurons. *J. Neurosci.*, **8**, 3733–3741.

GASIC, G.P. & HOLLMANN, M. (1992). Molecular neurobiology of glutamate receptors. *Annu. Rev. Physiol.*, **54**, 507–536.

GEOFFROY, M., LAMBOLEZ, B., AUDINAT, E., HAMON, B., CREPEL, F., ROSSIER, J. & KADO, R.T. (1991). Reduction of desensitization of a glutamate ionotropic receptor by antagonists. *Mol. Pharmac.*, **39**, 587–591.

GU, Y. & HUANG, L.-Y.M. (1991). Block of kainate receptor channels by Ca^{2+} in isolated trigeminal neurons of rat. *Neuron*, **6**, 777–784.

HATEFI, Y. & HANSTEIN, W.G. (1969). Solubilization of particulate proteins and non-electrolytes by chaotropic agents. *Biochemistry*, **62**, 1129–1136.

HONORE, T. & DREJER, J. (1988). Chaotropic ions affect the conformation of quisqualate receptors in rat cortical membranes. *J. Neurochem.*, **51**, 457–461.

HONORE, T. & NIELSEN, M. (1985). Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.*, **54**, 27–32.

HONORE, T., DREJER, J. & NIELSEN, M. (1986). Calcium discriminates two [^{3}H] kainate binding sites with different molecular target sizes in rat cortex. *Neurosci. Lett.*, **65**, 47–52.

JAHR, C.E. & STEVENS, C.F. (1987). Glutamate activates multiple single channel conductances in hippocampal neurons. *Nature*, **325**, 522–525.

JOHNSON, J.W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurones. *Nature*, **325**, 529–531.

KEINANEN, K., WISDEN, W., SOMMER, B., WERNER, P., HERB, A., VERDOORN, T.A., SAKMANN, B. & SEEBURG, P.H. (1990). A family of AMPA-selective glutamate receptors. *Science*, **249**, 556–560.

KISKIN, N.I., KRISHTAL, O.A., TSYNDRENKO, A.Y. & AKAIKE, N. (1986). Are sulphydryl groups essential for function of the glutamate-operated receptor ionophore complex. *Neurosci. Lett.*, **66**, 305–310.

KRISHTAL, O.A. & PIDOPLICHKO, V.I. (1980). A receptor for protons in the nerve cell membrane. *Neuroscience*, **5**, 2325–2327.

LAMBOLEZ, B., AUDINAT, E., BOCHET, P., CREPEL, F. & ROSSIER, J. (1992). AMPA receptor subunits expressed by single Purkinje cells. *Neuron*, **9**, 247–258.

MAYER, M.L. & VYKLICKY, L. (1989). Concanavalin A selectively reduces desensitization of mammalian neuronal quisqualate receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 1411–1415.

MAYER, M.L., VYKLICKY, L. & WESTBROOK, G.L. (1989). Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurones. *J. Physiol.*, **415**, 329–350.

MAYER, M.L., WESTBROOK, G.L. & GUTHRIE, P.B. (1984). Voltage dependent block by Mg^{2+} of NMDA responses in spinal cord neurons. *Nature*, **309**, 261–263.

MAYER, M.L., WESTBROOK, G.L. & VYKLICKY, L. (1988). Sites of antagonist action on N-methyl-D-aspartic acid receptors studied using fluctuation analysis and a rapid perfusion technique. *J. Neurophysiol.*, **60**, 645–663.

MCDONALD, J.W. & JOHNSTON, M.V. (1990). Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res. Rev.*, **15**, 41–70.

MONAGHAN, D.T., NGUYEN, L. & COTMAN, C.W. (1986). The distribution of [^{3}H] kainate sites in primate hippocampus is similar to the distribution of both Ca^{2+}-sensitive and Ca^{2+}-insensitive [^{3}H] kainate binding sites in rat hippocampus. *Neurochem. Res.*, **11**, 1073–1082.

MURPHY, D.E., SNOWHILL, E.W. & WILLIAMS, M. (1987). Characterisation of quisqualate recognition sites in rat brain tissue using DL-[^{3}H]-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and a filtration assay. *Neurochem. Res.*, **12**, 775–782.

NIELSEN, E.O., CHA, J.-H.J., HONORE, T., PENNEY, J.B. & YOUNG, A.B. (1988). Thiocyanate stabilizes AMPA binding to the quisqualate receptor. *Eur. J. Pharmacol.*, **157**, 197–203.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBERT, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*, **307**, 462–465.

O'DELL, T.J. & CHRISTENSEN, B.N. (1989). Horizontal cells isolated from catfish retina contain two types of excitatory amino acid receptors. *J. Neurophysiol.*, **61**, 1097–1109.

OLSEN, R.W., SZAMRAJ, O. & HOUSER, C.R. (1987). [^{3}H]AMPA binding to glutamate receptor sub-populations in rat brain. *Brain Res.*, **402,** 243–254.

PATNEAU, D.K. & MAYER, M.L. (1990). Structure-activity relationships for amino acid transmitter candidates acting at N-methyl-D-aspartate and quisqualate receptors. *J. Neurosci.*, **10,** 2385–2399.

PATNEAU, D.K. & MAYER, M.L. (1991). Kinetic analysis of interactions between kainate and AMPA: evidence for activation of a single receptor in mouse hippocampal neurons. *Neuron*, **6,** 785–798.

PEROUANSKY, M. & GRANTYN, R. (1989). Separation of quisqualate- and kainate-selective glutamate receptors in cultured neurons from the rat superior colliculus. *J. Neurosci.*, **9,** 70–80.

PETERS, S., KOH, J. & CHOI, D.W. (1987). Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurons. *Science*, **236,** 589–593.

RANDLE, J.C.R., VERNIER, P., GARRIGUES, A.-M. & BRAULT, E. (1988). Properties of the kainate channel in rat brain mRNA injected Xenopus oocytes: ionic selectivity and blockage. *Mol. Cell. Biochem.*, **80,** 121–132.

RASSENDREN, F.A., LORY, P., PIN, J.P. & NARGEOT, J. (1990). Zinc has opposite effects on NMDA and non-NMDA receptors in Xenopus oocytes. *Neuron*, **4,** 733–740.

SAWYER, W.H. & PUCKERIDGE, J. (1973). The dissociation of proteins by chaotropic salts. *J. Biol. Chem.*, **248,** 8429–8433.

SHAHI, K. & BAUDRY, M. (1992). Increasing binding affinity of agonists to glutamate receptors increases synaptic responses at glutamatergic synapses. *Proc. Natl. Acad. Sci. U.S.A.*, **89,** 6881–6885.

SMART, T.G. (1992). A novel modulatory binding site for zinc on the $GABA_A$ receptor complex in cultured rat neurones. *J. Physiol.*, **447,** 587–625.

SMART, T.G. & CONSTANTI, A. (1982). A novel effect of zinc on the lobster muscle GABA receptor. *Proc. R. Soc. B.*, **215,** 327–341.

SOMMER, B., KEINANEN, K., VERDOORN, T.A., WISDEN, W., BURNASHEV, N., HERB, A., KOHLER, M., TAKAGI, T., SAKMANN, B. & SEEBURG, P.H. (1990). Flip and flop: a cell-specific functional switch in glutamate-operated channels in the CNS. *Science*, **249,** 1580–1585.

TANG, C.M., DICHTER, M. & MORAD, M. (1989). Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. *Science*, **243,** 1474–1477.

TANG, C.M., DICHTER, M. & MORAD, M. (1990). Modulation of the N-methyl-D-aspartate channel by extracellular H^+. *Proc. Natl. Acad. Sci. U.S.A.*, **87,** 6445–6449.

THIO, L.L., CLIFFORD, D.B. & ZORUMSKI, C.F. (1991). Characterisation of quisqualate receptor desensitization in cultured rat hippocampal neurons. *J. Neurosci.*, **11,** 3430–3441.

TRAYNELIS, S.F. & CULL-CANDY, S.G. (1990). Proton inhibition of N-methyl-D-aspartate receptors in cerebellar neurons. *Nature*, **345,** 347–350.

TRAYNELIS, S.F. & CULL-CANDY, S.G. (1991). Pharmacological properties and H^+ sensitivity of excitatory amino acid channels in rat cerebellar granule neurones. *J. Physiol.*, **433,** 727–763.

TRUSSELL, L.O., THIO, L.L., ZORUMSKI, C.F. & FISCHBACH, G.D. (1988). Rapid desensitization of glutamate receptors in vertebrate central neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **85,** 2834–2838.

UMBACH, J.A. & GUNDERSEN, C.B. (1989). Mercuric ions are potent noncompetitive antagonists of human brain kainate receptors expressed in Xenopus oocytes. *Mol. Pharmacol.*, **36,** 582–588.

VERDOORN, T.A. & DINGLEDINE, R. (1988). Excitatory amino acid receptors expressed in *Xenopus* oocytes: agonist pharmacology. *Mol. Pharmacol.*, **34,** 298–307.

VLACHOVA, V., VYKLICKY, L., VYKLICKY, L.Jr. & VYSKOCIL, F. (1987). The action of excitatory amino acids on chick spinal cord neurons in culture. *J. Physiol.*, **386,** 425–438.

VYKLICKY, L., VLACHOVA, V. & KRUSEK, J. (1990). The effect of external pH changes on responses to excitatory amino acids in mouse hippocampal neurones. *J. Physiol.*, **430,** 497–517.

VYKLICKY, L., VYKLICKY, L.Jr., VYSKOCIL, F., VLACHOVA, V., UJEC, E. & MICHEL, J. (1986). Evidence that excitatory amino acids not only activate the receptor channel complex but also lead to use-dependent block. *Brain Res.*, **363,** 148–151.

WESTBROOK, G.L. & MAYER, M.L. (1987). Micromolar concentrations of Zn^{2+} antagonise NMDA and GABA responses of hippocampal neurons. *Nature*, **328,** 640–643.

ZORUMSKI, C.F. & YANG, J. (1988). AMPA, kainate, and quisqualate activate a common receptor-channel complex on embryonic chick motoneurons. *J. Neurosci.*, **8,** 4277–4286.

(Received October 13, 1992
Revised February 23, 1993
Accepted March 1, 1993)