Eur Biophys J (1990) 19:63–68

European Biophysics Journal

Kainate activated single channel currents as revealed by domoic acid

M. Sciancalepore [1], Z. Gałdzicki [2], X. Zheng [1], and O. Moran [1]

[1] Scuola Internazionale Superiore di Studi Avanzati, Strada Costiera 11, I-34014 Trieste, Italy
[2] Department of Biophysics, Academy of Medicine, ul. Chałubinskiego 10, PL-50 368 Wrocław, Poland

Received June 8, 1990/Accepted September 4, 1990

Abstract. We have studied the properties of kainic acid receptor-activated channels using domoic acid as an agonist. Similarities of the electrophysiological, pharmacological and noise properties of domoic acid and kainic acid-evoked currents confirm that domoate is a potent and specific agonist of the kainate receptor. Single-channel properties of domoic acid-evoked currents were directly determined from outside-out membrane patches for the first time, and results were compared with those obtained by fluctuation analysis of macroscopic currents. Small conductance cationic-selective channels of ≈ 4 pS and a mean open time of 2 to 3 ms were detected using both methods.

Key words: Domoic acid – Kainic acid – Excitatory aminoacids – Patch-clamp

Introduction

Glutamic acid is widely accepted to be the main mediator of fast excitatory neurotransmission in mammalian central neurones. Three major classes of glutamate receptors have been identified from their pharmacological and biophysical characterization, and named after their selective agonists: N-methyl-D-aspartate (NMDA), quisqualic acid (QA) and kainic acid (KA) (Watkins and Evans 1981; Mayer and Westbrook 1987). A particular property of the NMDA receptor subtype is the voltage-dependent extracellular Mg^{2+} blockage of its electrical response, while KA and QA are characterized by their extracellular Mg^{2+} insensitivity (Mayer et al. 1984; Nowak et al. 1984). This property has been extensively used to classify glutamic acid receptors as Mg^{2+}-sensitive (NMDA) and Mg^{2+}-insensitive (non NMDA).

While there is a good knowledge of the NMDA receptor-channel complex, less is known about the properties of the channels correlated to non-NMDA receptors. The single channel properties of the KA-activated channels could be investigated only by using methods of fluctuation analysis (Ascher and Nowak 1988 a; Cull-Candy and Usowicz 1987; Cull-Candy et al. 1988). In this work we are reporting, for the first time in our knowledge, a direct observation of the single-channel events associated with the KA receptor. This could be achieved using domoic acid (DA), which is a potent agonist of the KA receptor (Biscoe et al. 1975; Debonnel et al. 1989 a, b; Hollmann et al. 1989; Verdoorn and Dingledine 1988).

Offprint requests to: O. Moran

Methods

Cell culture

Experiments were performed on primary culture of cerebellar granule cells from eight day old Wistar rats as previously described (Levi et al. 1984). Electrophysiological experiments were done between 5 and 12 days in culture in order to avoid variability in results during the cell development in vitro (Sciancalepore et al. 1989).

Electrophysiology

Ionic currents from outside-out and whole-cell configurations (Hamill et al. 1981) were recorded with a standard patch-clamp amplifier (EPC-7, List Medical Instruments) and stored on video recorder tapes for later analysis.

The bath solution contained (in m*M*): 130 NaCl, 3 KCl, 1.5 $CaCl_2$, 10 HEPES-NaOH, 6 *d*-glucose and 10 tetraethylammonium chloride. Tetrodotoxin 1 μM was added to the external solution to block sodium channels. The pipette solution, dialyzing the intracellular compartment, was (in m*M*): 150 CsF, 1 EGTA, 10 HEPES-KOH, 6 *d*-glucose. In both solutions pH was adjusted to 7.4. In some experiments CsCl was used instead of CsF and no significant differences were observed in the electrophysiological results. Glutamic acid, NMDA, and KA were

purchased from Sigma (USA). Domoic acid was purchased from Diagnostic Chemical Ltd. (Canada). Experiments were performed at room temperature (22–24 °C).

Macroscopic currents

Uncoated and fire polished 5–8 MΩ borosilicate glass pipettes (Hilgemberg) were used to record currents in the whole-cell configuration. Agonists were perfused by pressure ejection for a period of 1 to 30 s to obtain macroscopic currents. Cells were always maintained under continuous flow of bath solution to avoid accumulation of agonists in the experimental chamber. Macroscopic currents were recorded at applied membrane potentials of −90 to +30 mV.

Single-channel currents

Single-channel currents were recorded from membrane patches in the outside-out configuration using Sylgard (Corning) coated fire polished 8–12 MΩ borosilicate glass pipettes. Agonists were directly applied to the bath solution by continuous gravity perfusion and data were acquired for several minutes; only patches that did not show spontaneous current fluctuations before the gravity perfusion of the agonist were considered for later analysis.

Data analysis

Data stored on magnetic tapes were filtered and transferred to an Atari (1040 ST) microcomputer, using a 12 bit A/D converter (M2 LAB, Instrutech) at a sampling interval of 200 to 500 μs or displayed on a chart recorder.

Single channel records were filtered with a 4-pole Bessel filter (4302, Ithaco) at a cut-frequency of 1 to 5 kHz, and were analysed with the THAC program (Sigworth 1983), developed for the Atari microcomputer (TAC, Instrutech). Dwell time distributions of single channel currents were fitted according to Sigworth and Sine (1987).

Macroscopic current records for noise analysis were filtered with a Butterworth filter (Krohn-Hite 3220) at a cut-off frequency of 5 kHz, and were acquired at 2 kHz. Spectral analysis of current noise evoked by agonists were constructed as described by Cull-Candy et al. (1988). Current signal was collected from long duration (>20 s) agonist application and the power spectrum was estimated using the fast Fourier transform algorithm. Power spectra obtained from control records, before the agonist application, were subtracted from those obtained from the agonist evoked currents. Resulting power spectra were fitted with a single or a double Lorentzian function. The fitted Lorentzian function ($S(f)=S(0)/(1+(f/f_c)^2)$) was extrapolated to zero frequency, to estimate the amplitude of single events as $i=(s(0)\,f_c\,\pi/2)/\langle i\rangle$ (Conti and Wanke 1975).

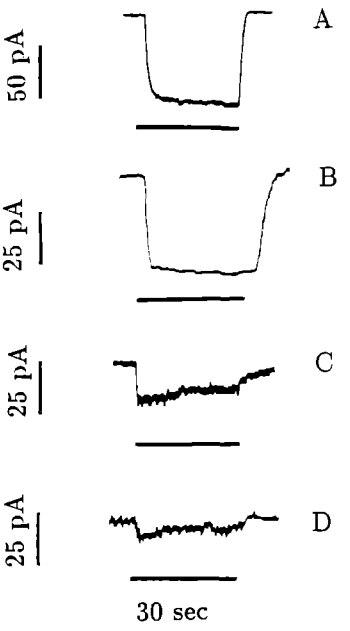

Fig. 1 A–D. Macroscopic currents evoked by glutamate receptor agonists, measured in whole-cell configuration, at an applied membrane potential of −70 mV. Agonists were perfused by 30 s pressure ejection, as indicated on bars under each record. The agonists tested were **A** domoic acid 25 μM; **B** kainic acid 50 μM; **C** glutamic acid 25 μM; and **D** NMDA 25 μM

Results

Whole-cell recording

Extracellular perfusion of DA (25–50 μM) in cerebellar granule cells by pressure ejection, in nominally Mg^{2+} free extracellular solutions, evoked macroscopic DA currents in 100% of the cases (Fig. 1 A). Macroscopic currents were also evoked by the application of KA, glutamate and NMDA (Fig. 1 B, C, D). The amplitude of these currents varied from cell to cell, being also correlated with the time in culture.

The amplitude of currents evoked by KA and DA were always larger than those evoked by the application of NMDA or glutamate (4–8 fold using the same agonist concentration). They had a rapid onset reaching a stable level that lasted for as long as the agonist was present, and decayed rapidly at the end of the agonist application (Fig. 1 A, B). No desensitization was observed when KA and DA were tested, even at concentrations as high as 1 m*M*. In contrast, glutamate and NMDA-evoked currents tended to decrease during the agonist application (Fig. 1 C, D) suggesting that the receptor tended to desensitize.

DA always evoked larger currents than those recorded upon KA application, as was observed when both agonists were applied at the same concentration on the same cell, using double barrel perfusion pipettes. On the average, the amplitude of currents evoked by the application of DA (10 μM) was found to be 4–5 fold higher than those evoked by the same concentration of KA. DA- and KA-evoked currents were not affected by the external application of amino-phosphonovaleric acid (APV;

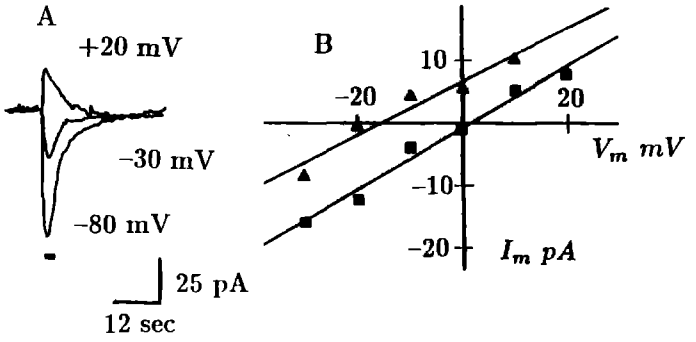

Fig. 2. **A** Currents activated by 1 s perfusion of 25 μM DA at different applied membrane potentials. **B** Current-voltage relationship obtained in the standard solutions (squares) and in an external solution in which NaCl was reduced to 50 m*M* (triangles). The reduction of the extracellular ion concentration produces a shift of the reversal potential of 17 mV to the left, which is consistent with a cationic current

100 μM), and were partially suppressed in the presence of kynurenic acid (300–400 μM reduce the peak current to 30–40%).

The amplitude of the DA-evoked current was proportional to the applied membrane potential (Fig. 2A). The current-voltage relationship of the DA evoked currents in our standard conditions (concentration of total anions and total cations nearly equal in internal and external solutions) showed a reversal potential around 0 mV (Fig. 2B), and in some cases presented an outward rectification at negative potentials. However, this rectification did not increase when Mg^{2+} (5–10 m*M*) was added to the external solution, as expected for the NMDA-activated currents (Ascher and Nowak 1988b; Mayer et al. 1984). Reducing the extracellular ion concentration, setting the NaCl to 50 m*M* by substituting iso-osmotically with glucose, the reversal potential shifted by about −20 mV (Fig. 2B). This shift in the reversal potential corresponds to a permeability ratio of positive and negative ions of 15, calculated according to the Goldman-Hodgkin-Katz equation. This result is consistent with the cationic selectivity already reported for the glutamic acid family activated channels (Ascher et al. 1988).

Noise analysis

In the whole-cell configuration, the increase of membrane macroscopic current that occurred upon the application of DA or KA was accompanied by an increase in the noise of the current signal. The magnitude of the signal to noise ratio of the KA- and DA-evoked macroscopic currents was less than those observed in the NMDA or glutamic acid-activated currents (see Fig. 1). Power spectra (in the range of 10 to 1000 Hz) of currents evoked by DA were constructed in order to have an estimate of the microscopic properties that underlie the agonists-activated currents. Most of the evaluated power spectra were well fitted by a single Lorentzian function. In some cases power spectra were better fitted using a double Lorentzian function. Examples of spectra obtained with DA and KA are shown in Fig. 3A, C.

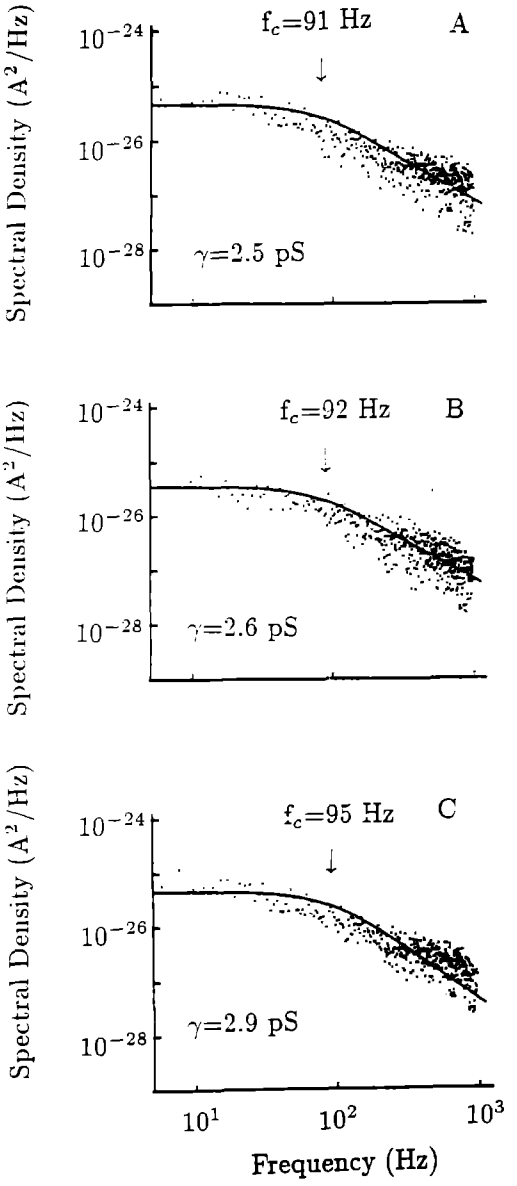

Fig. 3A–C. Power spectra obtained from DA and KA evoked macroscopic currents measured at −70 mV. Smooth lines were obtained by fitting spectra with a Lorentzian function. The cut-off frequency f_c, and the conductance of the unitary events γ are indicated on the figure. Spectra were obtained from currents evoked by 25 μM DA in nominally Mg^{2+} free solutions (**A**), and in the presence of extracellular 5 m*M* Mg^{2+} (**B**). A spectrum obtained in presence of 25 μM KA is also presented (**C**)

The analysis of the spectral density of the DA-evoked currents revealed a characteristic cut-off frequency (f_c) of 98±26 Hz (n=10), which corresponds to a time constant τ_{noise} of 1.74±0.34 ms. When a double Lorentzian was used to fit the power spectra, a slow frequency component of about 55 Hz and a component faster than 380 Hz were determined. A similar f_c was also found on KA-activated currents, where the estimated value of f_c=156±81 Hz (n=7) which corresponds to τ_{noise}=1.30±0.57 ms is not statistically different from that observed for the DA-evoked currents.

The amplitude of unitary events associated with the current noise were calculated from the extrapolation of

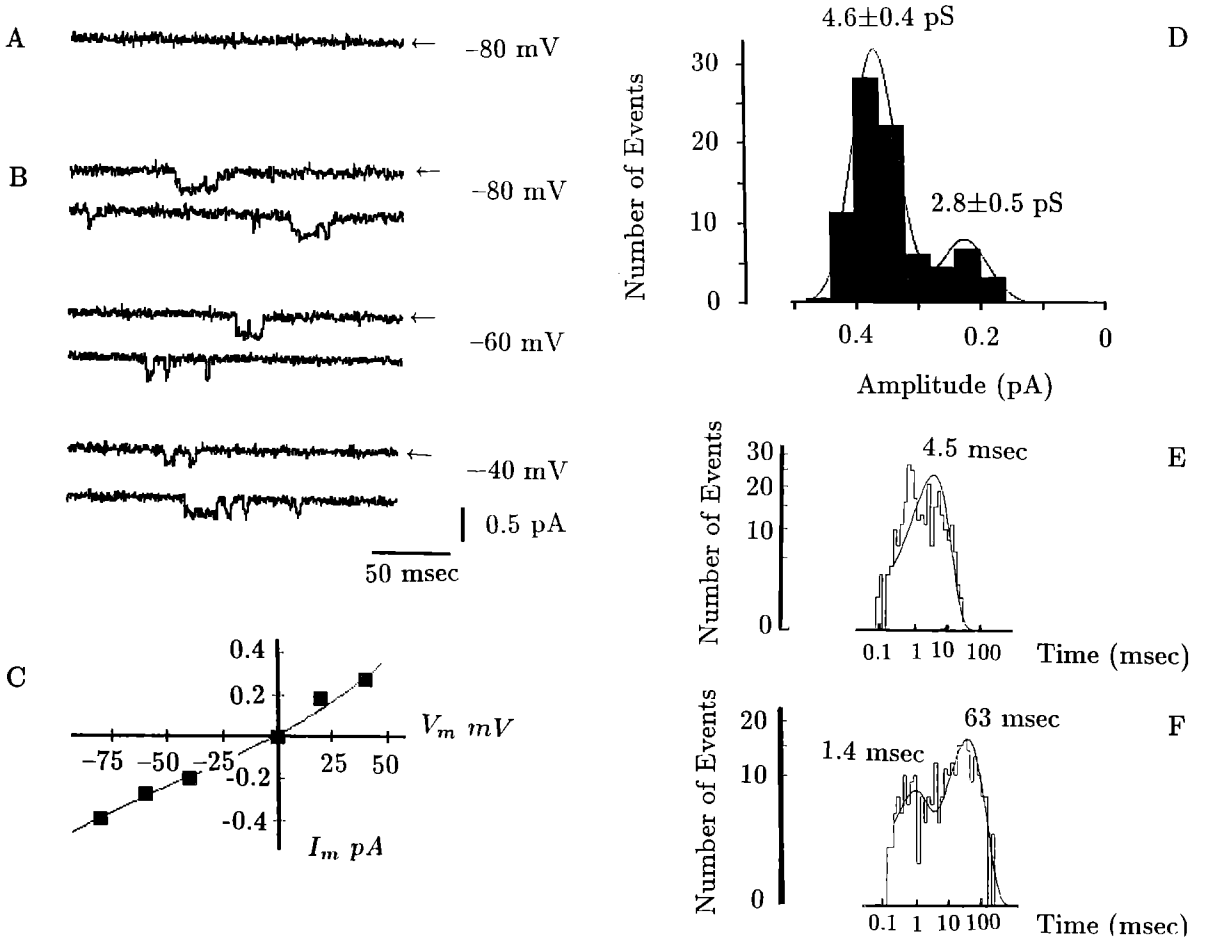

Fig. 4 A–F. Single channel currents recorded before the application of the agonist (**A**) and during the perfusion of the outside-out membrane patch with 10 μM DA (**B**). Records were filtered at 1 kHz. The applied membrane potential is indicated on the right of the records. Single-channel current-potential relationship (**C**) shows a reversal potential near to zero. The current amplitude histogram (**D**), open time histogram (**E**) and shut time histogram (**F**) were constructed from single-channel records obtained at an applied membrane potential of −80 mV

the Lorentzian function to zero frequency (see Cull-Candy et al. 1988). Power spectra of DA induced noise, estimated from 10 different cells, gave an amplitude value that corresponds to a conductance of 3.5±0.8 pS, as measured at applied membrane potentials between −30 and −70 mV. The conductance value was independent on the applied membrane potential. Single channel conductance for KA-evoked currents evaluated by spectral analysis of macroscopic currents was very similar to that of DA-evoked channels (Fig. 3C).

In some experiments, the noise was analysed in the presence of 5 m*M* Mg^{2+} in the external solution during the stimulation of the cell with the agonist. We did not observe any significant difference of the noise characteristics of the DA or KA evoked currents, as expected for the non-NMDA activated currents (Fig. 3B).

Single-channel recording

DA (10 μM) evoked single-channel currents in outside-out patches when applied to the bath solution, as is shown in Fig. 4A and B. A record, without any significant current fluctuation, obtained before the DA application in the same patch, is also shown as control. An accurate analysis of the DA-activated single channel currents could be done only on 7 patches out of 52, because of the short duration and the small amplitude of the events.

Single-channel currents observed after the application of DA to outside-out patches corresponded to two populations of conductances, with values of 2.4±0.4 pS and 4.4±0.3 pS (mean±s.d.; $n=7$) as revealed by the amplitude histograms constructed for each experiment (Fig. 4D). The same conductance values were also observed in a few cell-attached experiments, in which the pipette filling solution contained 5–20 μM DA. Higher conductance values, of 7 pS and 50 pS, were also observed very rarely in a few patches (data not shown). The current-voltage (I–V) relation of the single-channel currents showed a reversal potential around 0 mV with a slight rectification at negative potentials (Fig. 4C), which is consistent with that observed on whole-cell experiments.

Dwell time distributions of DA-activated channels were studied on seven successful excised patches. DA-activated channels tended to be distributed in periods of

activity, followed by silent periods. The measured mean open time was 3.5 ± 1.5 ms. This value was consistent with the open time distribution, which was quite well fitted by a single exponential function with a time constant τ_{open} of 3.3 ± 1.8 ms (Fig. 4 E). The shut time distribution revealed two different close time populations. The fast closure, with a time constant of 4.0 ± 2.6 ms, corresponded to the closure which occurred during the activity periods, while a slow closure, with time constant longer than 60 ms, was correlated with the time between successive groups of activity (Fig. 4 F). Open channel probability measured in outside-out patches showed very small values ($P_0 \leq 0.06$). Neither open channel probability nor dwell time distribution of DA-activated channels showed any significant voltage-dependence.

Discussion

Enzymatically dissociated cerebellar granule cells were found to have glutamate receptors and were tested for responsiveness to glutamate as well as to the specific agonists of its subtypes, NMDA and KA. Electrophysiological responses were characteristically dependent on the agonists tested, that is consistent with previous data obtained in explant granule cell culture (Cull-Candy et al. 1988).

DA is a structural analog of KA and it is naturally present in several phytoplankton species (Takemoto and Daigo 1960). The excitatory effect of DA has been demonstrated in rat and frog spinal cord by intracellular recording (Biscoe et al. 1975; Shinosaki and Ishida 1976). Indeed, DA was observed to act as a potent agonist of the KA receptor-channel complex expressed from exogenous cDNA in *Xenopus* oocytes (Hollmann et al. 1989). Recently, more attention has been paid to DA because it was considered responsible for alimentary intoxication events which occurred in Canada during 1987 (Glavin et al. 1989; Debonnel et al. 1989 a, b).

We have shown that DA evoked low noise and magnesium insensitive macroscopic cationic currents, which can be partially blocked by kynurenic acid and are insensitive to APV. This property, as well as the microscopic properties of these currents estimated by fluctuation analysis are similar to those found in currents evoked by KA. This characteristic favours the hypothesis that DA activates the same non-NMDA receptor as KA does. Similarity between DA and KA receptors can be also suggested from three other main arguments: 1 – Part of the DA chemical structure is identical to that of KA (Biscoe et al. 1975); 2 – DA evokes currents in *Xenopus* oocytes expressing brain mRNA (Verdoorn and Dingledine 1988) or KA receptor cDNA (Hollmann et al. 1989); 3 – DA strongly binds KA receptors extracted from solubilized brain membranes (Hampson and Wenthold 1988).

We found some difficulty in obtaining acceptable single channel records of DA-activated currents because of the very small amplitude of the single channel events. In fact, we had to discard more than 80% of the patches because we could not resolve the single channel events. This limitation prevented us from obtaining good statistics on the frequency of appearance of DA-activated channels in different cells (as an estimate of channel density), because it is quite difficult to say if the events were not observed because of the unfavorable signal to noise ratio, or because DA-activated channels were absent.

We were able to identify a homogeneous population of single DA activated channels at applied membrane potentials from -90 to $+30$ mV, corresponding to conductances of 4 pS and 2.4 pS. However, the most frequent ($>80\%$) conductance value was 4.4 pS, that argues in favour of the notion that the 2.4 pS value may be a subconductance state of the channel. On the other hand, if the 4.4 pS conductance found had been a superposition of two 2.4 pS events, this former conductance should have been found less frequently. In fact, the conductance of 3.5 pS estimated by noise analysis lies between the minimum and the maximum values of those found by single-channel recording, as expected from the estimation of the weighted average of conductances of the unitary events. A remarkable correspondence between the τ_{open} measured directly from single-channel recording and τ_{noise} was also found. This value is consistent with those calculated from noise analysis of KA-evoked macroscopic currents by others (Ascher and Nowak 1988 a; Cull-Candy and Usowicz 1987; Cull-Candy et al. 1988). This uniformity on conductance data, as well as kinetic results, coming from different methods of measurement such as single-channel recording and macroscopic noise analysis, confirms the goodness of these observations.

Previous attempts to obtain single-channel records using KA as the agonist for the glutamate receptor subtype (Ascher et al. 1988; Cull-Candy et al. 1988) resulted in events of higher conductance than expected, probably due to a non-specific activation of glutamate receptor subtypes other than KA. In contrast, the use of DA as agonist seems to evoke preferentially only one type of channel (4 pS), and very rarely other conductance channels. Thus, this unspecificity of activation by KA, seems to be superseded when DA is applied, and can be related to a major affinity of DA for KA receptors, and this is suggested by the higher potency of DA found here and in other preparations (Hollman et al. 1989; Verdoorn and Dingledine 1988).

Selectivity of the DA activated channels has shown to be cationic, as expected for a typical glutamic acid receptor associated current (Asher and Nowak 1988 b). The rectification of inward currents observed on whole-cell experiments was also seen in single channel current records. This characteristic has been previously reported in KA-activated currents by Ascher and Nowak (1988 a). We tried to explain this rectification as an effect similar to that observed for the interaction of Mg^{2+} with the NMDA-activated channel. However, when extracellular Mg^{2+} was added no significant voltage-dependent effects were observed on the DA-activated macroscopic current amplitude or on the single-channel properties measured by noise analysis. Nevertheless, more experiments on the particular selectivity of the DA-activated channels to each ionic species, and the possible interaction of the channel with ions different than Mg^{2+} are in progress.

Acknowledgements. M.S. was partially supported by Fidia Research Foundation.

References

Ascher P, Nowak L (1988a) Quisqualate and kainate-activated channels in mouse central neurons in culture. J Physiol 399:227–245

Ascher P, Nowak L (1988b) The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurons in culture. J Physiol 399:247–266

Ascher P, Bregestovski P, Nowak L (1988) N-Methyl-D-aspartate-activated channels of mouse central neurons in magnesium-free solutions. J Physiol 399:207–226

Biscoe TJ, Evans RH, Headley PM, Martin M, Watkins JC (1975) Domoic and quisqualic acids as potent amino acid excitants of frog and rat spinal neurons. Nature 255:166

Conti F, Wanke E (1975) Channel noise in nerve membranes and lipid bilayers. Q Rev Biophys 8:451–506

Cull-Candy SG, Usowicz MM (1987) Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. Nature 325:525–528

Cull-Candy SG, Howe JR, Ogden DC (1988) Noise and single channels activated by excitatory amino acids in rat cerebellar granule neurons. J Physiol 400:189–222

Debonnel G, Beauchesne L, de Montigny C (1989a) Domoic acid, the alleged "mussel toxin", might produce its neurotoxic effect through kainate receptor activation: an electrophysiological study in the rat dorsal hippocampus. Can J Physiol Pharmacol 67:29–33

Debonnel G, Weiss M, de Montigny C (1989b) Reduced neuroexcitatory effect of domoic acid following mossy fiber denervation of the rat dorsal hippocampus: further evidence that toxicity of domoic acid involves kainate receptor activation. Can J Physiol Pharmacol 67:904–908

Glavin GB, Pinsky C, Bose R (1989) Mussel poisoning and excitatory amino acid receptors. Trends Pharm Sci 10:15

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Archiv 391:85–100

Hampson DR, Wenthold RJ (1988) A kainic acid receptor from frog brain purified using domoic acid affinity chromatography. J Biol Chem 263:2500–2505

Hollmann MA, O'Shea-Greenfield A, Rogers W, Heinemann S (1989) Cloning by functional expression of a member of the glutamate receptor family. Nature 342:643–648

Levi G, Aloisi F, Ciotti MT, Gallo V (1984) Autoradiographic localization and depolarization-induced release of acidic amino acids in differentiation granule cells cultures. Brain Res 290:77–86

Mayer ML, Westbrook GL (1987) The physiology of excitatory amino acid in the vertebrate central nervous system. Prog Neurobiol 28:197–276

Mayer ML, Westbrook GL, Guthric PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurons. Nature 309:261–263

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurons. Nature 307:462–465

Sciancalepore M, Forti L, Moran O (1989) Changes of N-Methyl-d-aspartate activated channels of cerebellar granule cells with days in culture. Biochem Biophys Res Comm 165:481–487

Shinozaki H, Ishida M (1976) Inhibition of quisqualate responses by domoic or kainic acid in crayfish opener muscle. Brain Res 109:435–439

Sigworth FJ (1983) An example of analysis in single-channel recording. Sakmann B, Neher E (eds). Plenum Press, New York, pp. 301–321

Sigworth FJ, Sine SM (1987) Data transformation for improved display and fitting of single channel dwell time histograms. Biophys J 52:1047-10

Takemoto T, Daigo K (1960) Über die Inhaltsstoffe von *Chondria armata* und ihre pharmakologische Wirkung. Archiv der Pharmazie 293:627–633

Verdoorn TA, Dingledine R (1988) Excitatory amino acid receptors expressed in *Xenopus* oocytes: agonist pharmacology. Mol Pharmacol 34:298–307

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Ann Rev Pharmacol Toxicol 21:165–204