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Neuroblastoma cells as possible model in the study of glutamate receptors

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Summary. The sensitivity of several cultured neuroblastoma cell lines to *L*-glutamate (Glu) and its analogues has been studied with the whole-cell patch clamp technique. Only in the neuroblastoma cell line N2A Glu induced a concentration dependent response consisting of an inward current. In addition, N2A cells responded to superfusion with kainate, quisqualate or *N*-methyl-*D*-aspartate, but to a lesser degree than to Glu. It is concluded that N2A cells could be useful as an *in vitro* model to study excitatory amino acid properties.

Keywords: Glutamic acid – Meuroblastoma – Voltage clamp – Excitatory amino acids

Neuroblastoma cells receive increasing interest as a model system in different research areas. Several neuroblastoma cell lines, each originating from a single cell that has been isolated from a spontaneously occurring or induced tumor of the nervous system, are now available and can be maintained in culture infinitely. When certain chemical compounds are added to the culture medium the cells start to differentiate. Upon differentiation the malignant character of the cells is lost and several properties of nerve cells come to expression. Differentiation of the cells is characterized by a decrease in growth rate, outgrowth of membrane processes, excitability, sensitivity to and release of neurotransmitters etc. Since isolated cells are easily obtained they are useful objects for experiments with the most advanced electrophysiological techniques. Recent studies have shown that the neuroblastoma cell line N1E-115 can be used as model for studying serotonin 5-HT3 [1] and neuronal type acetylcholine [2] receptor mediated ion current. Here it will be shown that glutamate induces a physiological response in a neuroblastoma cell line.

Materials and methods

Cells of the clones B35, B50 [6], NG108–15, Y79, N1E–115 and N2A were routinely grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 7.5% fetal calf serum and the following amino acids (in mM): cysteine · HCl 0.3, L-alanine 0.4, L-asparagine 0.45, L-aspartic acid 0.4, L-proline 0.4 and L-glutamic acid 0.4. Cells were cultured in a humidified atmosphere containing 5% CO_2 at 37°C. Differentiation was initiated 2–3 days after subculture by adding 1 mM dibutyrylcyclic-AMP and 1 mM 3-isobutyl-1-methylxanthine to the culture medium. Medium was refreshed every 2–3 days.

Membrane currents were measured with the patch-clamp technique in the whole-cell voltage clamp configuration. The fire polished patch electrodes had an internal diameter of 1.5–3 μm and a resistance of 1–3 MΩ. During voltage clamp experiments the membrane potential was held at −80 mV. Membrane currents were low-pass filtered, digitized by a transient recorder (8 bits; 1024 points/record) and stored on a magnetic disc for off-line computer analysis.

The pipette solution contained (in mM): 75 CsCl, 75 CsF, 10 EGTA, 10 Hepes, 10 sucrose (pH 7.2 with CsOH, osmolality 310 mOsm.). The external solution contained (in mM): 125 NaCl, 5.5 KCl, 1.8 $CaCl_2$, 5 glucose, 10 Na-Hepes, 10 Hepes, 53.7 sucrose (pH 7.4, osmolality 340 mOsm.). During an experiment a cell was continuously superfused with external solution containing known concentrations of the agonists L-glutamic acid (Glu), kainic acid (KA), quisqualic acid (QA) or N-methyl-D-aspartic acid (NMDA). Cells were superfused through a capillary of 1 mm diameter positioned at approximately 50–100 μm from the cell. Experiments were performed at room temperature (20–24°C). Concentration effect curves were fitted using a Levenberg-Marquardt non-linear least squares algorithm (Marquardt, 1963). Results are presented as mean ± s.d..

Results

Superfusion with Glu at a concentration of 5.10^{-5} M did not induce a response in cells of the clones B35 ($n = 9$), B50 ($n = 8$), NG108–15 ($n = 7$), Y79 ($n = 14$) and N1E–115 ($n = 29$). The same concentration of Glu induced an inward current in all cells of the clone N2A ($n > 50$). The amplitude of the Glu-induced response varied between 60 and 500 pA, dependent on cell size. The Glu-induced response was concentration dependent (Fig. 1). The minimum concentration of Glu to evoke a detectable inward current was 10^{-7} M and the maximum response was obtained with 10^{-4} M Glu. From the concentration-effect curve the estimated EC50 and slope factor were 11 ± 2 μM and 1.0 ± 0.1 respectively.

To study the sensitivity of these cells to agonists which are relative selective for sub-types of the Glu receptor, they were also superfused with KA, QA and NMDA ($5 \cdot 10^{-5}$ M). QA always induced an inward current ($n = 17$). Only 60% of the cells ($n = 16$) were responsive to NMDA application. Sensitivity to KA was apparent in 80% of the cells ($n = 20$) (Fig. 2). There was no significant difference in amplitude of the inward current induced by these Glu analogues. In 14 cells the KA-induced response consisted of a transient inward current followed by an outward current (Fig. 3). The maximum inward current induced by the analogues was 43% of the by Glu ($5 \cdot 10^{-5}$ M) induced current.

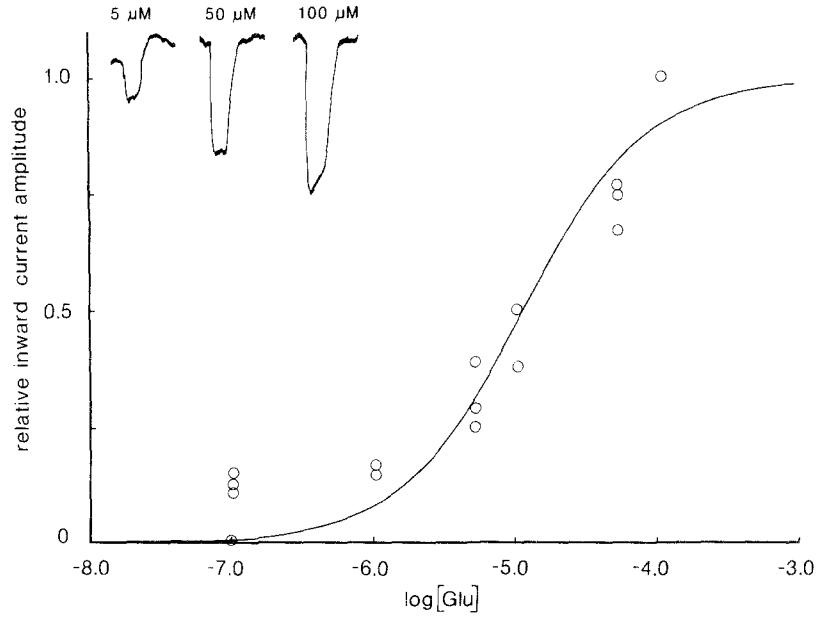

Fig. 1. Concentration-effect curve of Glu. Increasing concentrations of Glu induced inward currents of increasing magnitude (inset). The estimated EC50 and slope factor of the concentration-effect curve were 11 ± 2 μM and 1.0 ± 0.1 respectively (n = 6 cells). Ordinate represents the peak amplitude of the inward current normalized to the maximum current amplitude, which was obtained by 10^{-4} M Glu in every experiment. Data were fitted by the concentration-effect function: $i/i_{max} = 1/\{1 + (EC50/[Glu])^n\}$

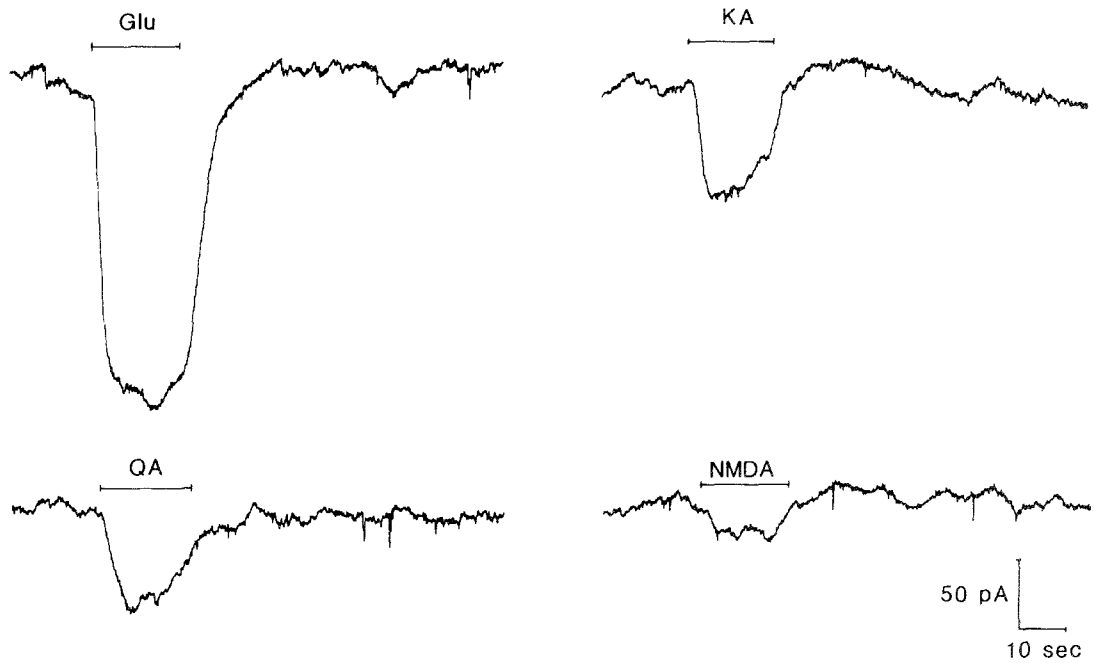

Fig. 2. Responses to superfusion with Glu receptor agonists. Application of 50 μM Glu, KA, QA or NMDA gave inward currents of different magnitudes

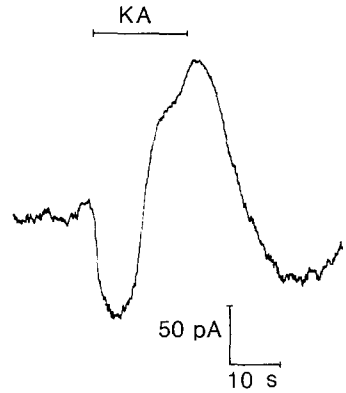

Fig. 3. A large part of the cells responded to KA application with a dual response consisting of a transient inward current followed by an outward current

Discussion

The primary objective of this study was to determine whether neuroblastoma cells can be used for physiological studies of Glu receptors. So far in excitatory amino acid research, neuroblastoma cell lines have only been used to study Glu receptor binding properties [3] and excitotoxicity [4]. This study shows that differentiated cells of the neuroblastoma cell line N2A are sensitive to application of Glu. The Glu response is an inward current and its amplitude is concentration dependent as has been found in various cell types [5]. The dual response to KA which was observed in most cells has not been reported before. Further experiments are being conducted to characterize the ionic currents involved in this response. This study suggest that neuroblastoma cells can be used as *in vitro* models for the study of excitatory amino acid receptor properties and associated ion channels.

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