ELSEVIER

Biochemical Pharmacology 64 (2002) 1195–1200

Biochemical Pharmacology

Commentary

Glutamate antagonists limit tumor growth

Wojciech Rzeski[a,*], Chrysanthy Ikonomidou[b], Lechoslaw Turski[c]

[a]*Department of Virology and Immunology, Institute of Microbiology and Biotechnology, Maria Curie-Sklodowska University, Akademicka 19, 20-033 Lublin, Poland*

[b]*Department of Pediatric Neurology, Charité, Virchow Clinics, Humboldt University, Augustenburger Platz 1, 13353 Berlin, Germany*

[c]*Solvay Pharmaceuticals Research Laboratories, C.J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands*

Abstract

The management of malignancies in humans constitutes a major challenge for contemporary medicine. Despite progress in chemotherapy, bone marrow transplantation, surgical measures, and radiation technologies, and in immunological and immunomodulatory approaches, humans continue to succumb to cancer due to tumor recurrence and metastatic disease. The excitatory neurotransmitter glutamate, which regulates proliferation and migration of neuronal progenitors and immature neurons during the development of the mammalian nervous system, is present in peripheral cancers. Since both neuronal progenitors and tumor cells possess propensity to proliferate and to migrate, and since glutamate and glutamate receptors are known to modify these phenomena in the nervous system, we proceeded to investigate the possible influence of glutamate antagonists on the proliferation and migration of tumor cells. We found and recently reported that glutamate *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) antagonists inhibit the proliferation of human colon adenocarcinoma, astrocytoma, breast and lung carcinoma, and neuroblastoma cells *in vitro*. The antiproliferative effect of glutamate antagonists is Ca^{2+}-dependent and results from decreased cell division and increased cell death. Glutamate antagonists produce morphological alterations in tumor cells, which consist of reduced membrane ruffling and pseudopodial protrusions, and decrease their motility and invasive growth. Furthermore, glutamate antagonists enhance *in vitro* cytostatic and cytotoxic effects of common chemotherapeutic agents used in cancer therapy. These findings demonstrate the anticancer potential of glutamate antagonists and suggest that they may be used as an adjunctive measure in the treatment of cancer.
© 2002 Elsevier Science Inc. All rights reserved.

Keywords: NMDA; AMPA; Tumor; Cytostatic; Antiproliferative; Migration; Metastasis

1. Introduction

Glutamate is an essential amino acid and a transmitter in the mammalian nervous system [1,2]. NMDA, AMPA, kainate, and metabotropic receptors are activated by glutamate [1,2]. Its neurotransmitter role was discovered about 50 years ago, when Hayashi [3,4] administered glutamate into the motor cortex of dogs and monkeys and triggered severe convulsions. At the same time, Lucas and Newhouse [5] made the observation that systemic administration of glutamate causes retinal degeneration in mice [5]. Further work linked activation of excitatory amino acid receptors to glutamate neurotoxicity and led to the concept of excitotoxicity [6–8]. Excitotoxicity was shown to mediate neuronal death in anoxic hippocampal cultures [9] and to be Ca^{2+}-dependent [10–12]. Glutamate receptor subtypes in mammalian brain sensitive to the agonists NMDA, AMPA, and kainate, and the quisqualate sensitive metabotropic site were identified, and antagonists that selectively block glutamate receptors were developed [11].

A new field of research emerged from these discoveries, attempting to explore the physiological functions of glutamate [11]. At the same time, substantial evidence was generated implicating abnormal glutamate signaling and excitotoxicity in the pathogenesis of various CNS diseases [11]. Now, a few decades after the discovery of the dual role of glutamate in the CNS, there is hardly any neurological or psychiatric disease entity in the pathogenesis of which glutamate has not been implicated. Glutamate has been pathogenetically linked to human psychiatric

* Corresponding author. Tel.: +48-81-537-5942; fax: +48-81-537-5959.
E-mail address: rzeskiw@biotop.umcs.lublin.pl (W. Rzeski).

Abbreviations: NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

0006-2952/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0006-2952(02)01218-2

disorders such as anxiety or depression and to neurological disorders such as epilepsy, spasticity, stroke, or traumatic brain injury [11–13]. Glutamate antagonists were demonstrated to have anxiolytic, anticonvulsant, muscle relaxant, sedative/anesthetic, and neuroprotective properties [11].

2. Glutamate in peripheral tissues

The landscape around glutamate research has changed somewhat in recent years, in that an increasing number of investigators have demonstrated the involvement of glutamate and glutamate signaling in non-neuronal tissues. Glutamate receptors have been identified in bone osteoblasts and osteoclasts, keratinocytes, megakaryocytes, pancreatic isle cells, the lung, the liver, the heart, kidney cells, adrenal tissue, and taste buds [14–17]. What role glutamate plays in peripheral tissues is unclear, and it remains a mystery whether glutamate-mediated signaling outside the CNS may ever become a valuable therapeutic target.

3. Establishing a link to cancer via the trophic functions of glutamate

Glutamate has trophic functions in the developing mammalian CNS. Glutamate and glutamate receptors are implicated in neuronal proliferation and migration during development, and glutamate receptors have been shown to critically regulate neuronal survival in the mammalian forebrain during a period of rapid growth termed ‘‘the brain growth spurt period’’ [18–20]. During that period, blockade of NMDA receptors was shown to trigger massive apoptotic neuronal death in the developing rodent brain [20,21]. This discovery provided the first insights into mechanisms that can damage the developing human brain in the fetal, neonatal, and early childhood periods. The pathogenesis of the fetal alcohol syndrome, a well-described neurodevelopmental disorder in humans exposed to alcohol during gestation, can be explained by this mechanism [21].

Certain characteristics of neuronal embryonic cells, including propensity to proliferate, migrate, and die, are shared by tumor cells, as is the regulation of their invasive behavior by trophic factors [16]. The above evidence, along with the knowledge that glutamate is present in non-neuronal tissues, including cancers, prompted us to investigate whether glutamate and glutamate antagonists may influence proliferation and migration of human cancer cells.

4. Glutamate antagonists and tumor growth

In a series of recently conducted experiments [22], we were able to demonstrate that glutamate antagonists inhibit division and migration, enhance death, and alter the morphology of tumor cells *in vitro*, resembling cytostatic drugs used in the therapy of cancer.

Eight different tumor cell lines were exposed to different concentrations of the NMDA antagonists (+)-dizocilpine (MK801), memantine, and ketamine or the AMPA antagonists 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI52466), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX), and 1-(4′-aminophenyl)-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one (CFM-2). Proliferation of tumor cells was decreased in cultures exposed to the antagonists in a concentration-dependent manner, whereas proliferation of human skin fibroblasts and bone marrow stromal cells was unaffected by this exposure (Fig. 1). Time–course studies revealed that the antiproliferative effect of glutamate antagonists was already established after 24 hr.

The effect of glutamate antagonists on tumor cell proliferation was attributed to both decreased cell division and increased cell death [22]. The threshold concentrations of dizocilpine or GYKI52466 required to elicit antiproliferative effects ranged between 1 and 50 μM. A significant antiproliferative effect of dizocilpine was detected at concentrations as low as 1 μM in colon adenocarcinoma cells and as low as 10 μM in astrocytoma cells. Similarly, GYKI52466 significantly inhibited the proliferation of colon adenocarcinoma cells at concentrations as low as 1 μM and of breast carcinoma cells at concentrations as low as 10 μM. Such concentrations of glutamate antagonists are required to modulate NMDA- or AMPA-mediated currents in non-neuronal tissues such as osteoblasts and osteoclasts [23,24] or to inhibit migration of embryonic cortical neurons [25].

Similar antiproliferative effects could be elicited by the NMDA antagonists ketamine and memantine and the AMPA antagonists NBQX and CFM-2 in lung carcinoma and rhabdomyosarcoma/medulloblastoma cells [22].

5. Glutamate antagonists and migration of cancer cells

Even more interesting than their antiproliferative action are the inhibition of migration and the morphological changes that glutamate antagonists produce in tumor cells. Inhibition of tumor cell migration, which is considered an indicator of antimetastatic action, can be achieved at much lower concentrations of glutamate antagonists than are required to slow proliferation. Limiting tumor metastasis is extremely important in cancer therapy, since metastatic disease and not local tumor growth determines mortality in most peripheral cancers. The opposite is the case in the treatment of CNS tumors, where antiproliferative action is of crucial importance in order to preserve neuronal tissue and function.

To evaluate the effect of glutamate antagonists on tumor cell morphology and migration, lung carcinoma,

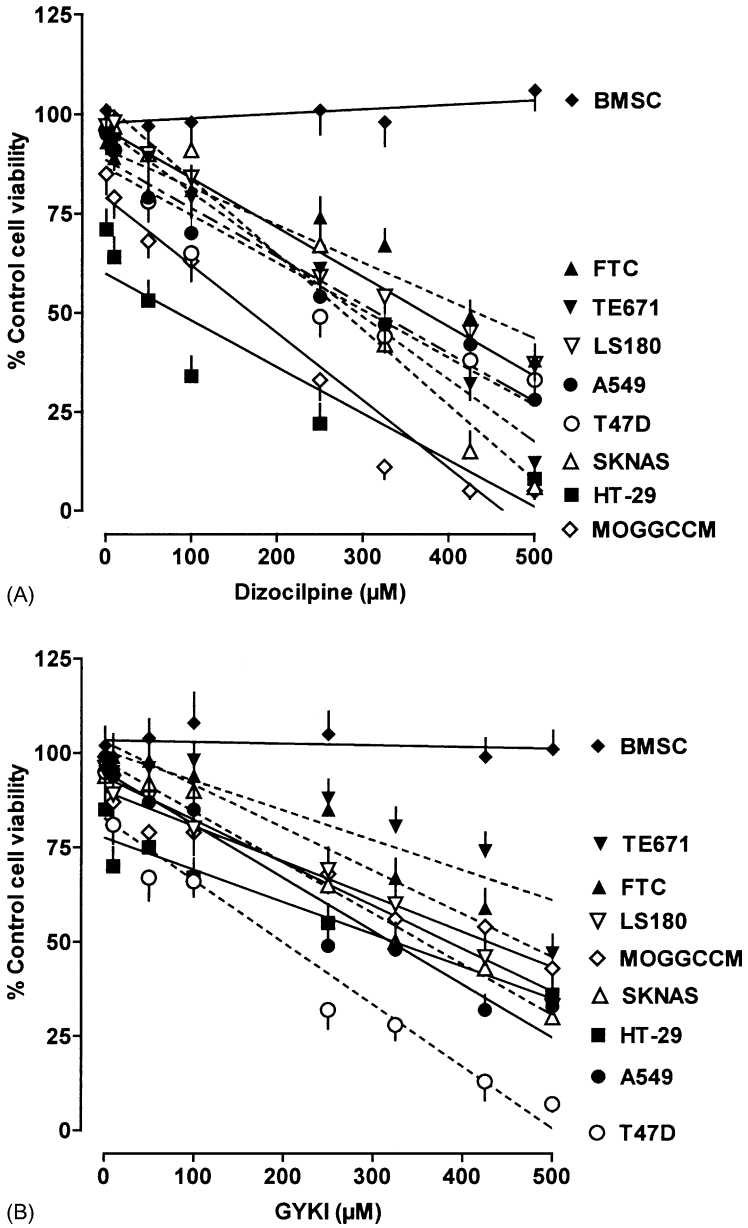

Fig. 1. Antiproliferative effects of glutamate antagonists in human cancer cells. The NMDA antagonist dizocilpine (A) and the AMPA antagonist GYKI52466 (B) exert a concentration-dependent antiproliferative effect in human tumor cell lines but not in human bone marrow stromal cells. Cells were exposed to either culture medium alone (control), dizocilpine (1–500 μM), or GYKI52466 (1–500 μM) over 96 hr, and viability was measured photometrically by means of the MTT assay. Data represent mean normalized optical densities ± SEM of 6–8 trials and were analyzed by means of linear regression. Abbreviations: SKNAS: human neuroblastoma; TE671: human rhabdomyosarcoma/medulloblastoma; MOGGCCM: human brain astrocytoma; FTC238: human thyroid carcinoma; A549: human Caucasian lung carcinoma; LS180: human Caucasian colon adenocarcinoma; T47D: human breast carcinoma; HT29: human colon adenocarcinoma; and BMSC: human bone marrow stromal cells.

rhabdomyosarcoma/medulloblastoma, and thyroid carcinoma cells were exposed to the NMDA antagonist dizocilpine (100 and 250 μM) or the AMPA antagonist GYKI52466 (100 and 250 μM) over 96 hr, and their morphology was examined by light and scanning electron microscopy. Light microscopy revealed that dizocilpine induced rounded cell appearance with numerous vacuoles in the cytoplasm, whereas exposure to GYKI52466 produced shrinkage of the cells and less prominent cytoplasmic vacuoles (Fig. 2). Electron microscopy revealed that tumor cells displayed an invasive phenotype with marked membrane ruffling and numerous pseudopodia, whereas tumor cells exposed to glutamate antagonists displayed a non-invasive phenotype with fewer pseudopodial protrusions [22].

To test whether glutamate antagonists may decrease tumor cell locomotion and invasiveness, lung carcinoma, rhabdomyosarcoma/medulloblastoma, and thyroid carcinoma cells were plated on polycarbonate membrane filters with a 3-μm pore size in the presence and absence of

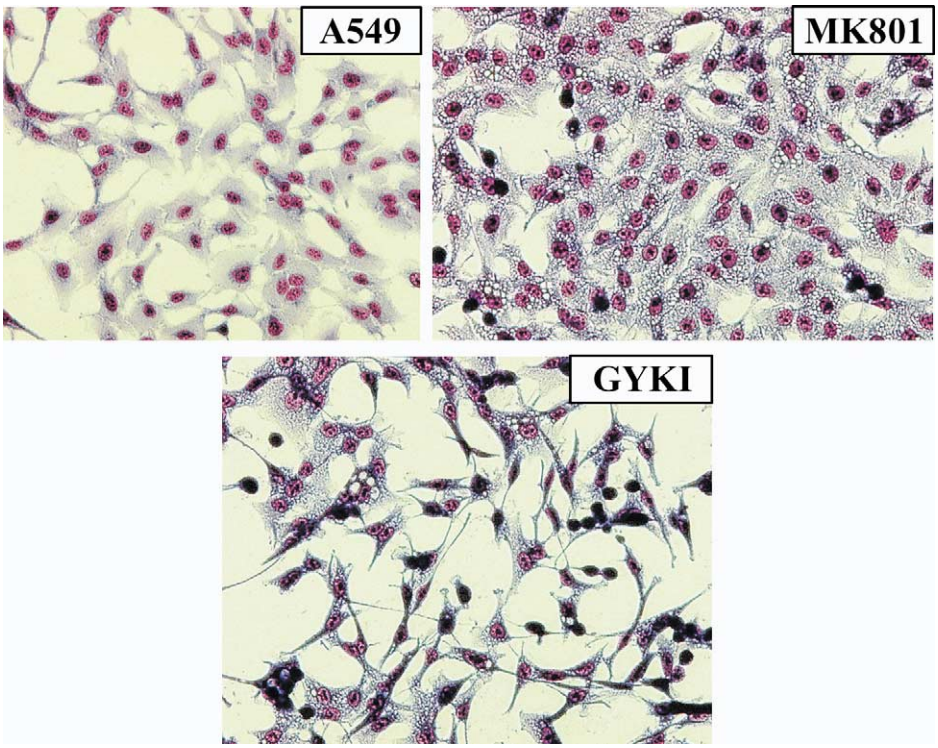

Fig. 2. Glutamate antagonists and tumor cell morphology. Light micrographs of lung carcinoma cells (A549) under control conditions and following exposure to dizocilpine (MK801; 250 μM) or GYKI52466 (250 μM). Tumor cells exposed to dizocilpine display multiple cytoplasmic vacuoles. GYKI-treated cells have an elongated appearance and less prominent cytoplasmic vacuolization.

dizocilpine or GYKI52466. In cultures exposed to glutamate antagonists, fewer cells migrated through the filters than occurred in the control cultures [22].

6. Synergistic effects of glutamate antagonists and cytostatic drugs

One important and clinically relevant finding of our studies is the synergistic effect of glutamate antagonists and common cytostatic agents used in cancer therapy. This finding implies that by combining glutamate antagonists with an existing chemotherapeutic regimen one might achieve superior cytostatic effects compared to either therapy alone [22].

We subjected lung carcinoma, astrocytoma, neuroblastoma, and rhabdomyosarcoma/medulloblastoma cells to treatment with the anticancer drugs cyclophosphamide, cisplatin, thiotepa, or vinblastine and either dizocilpine or GYKI52466. As expected, the cytostatic drugs decreased tumor cell viability in a concentration-dependent manner. This effect was enhanced in all of the cell lines tested by both (+)-dizocilpine and GYKI52466. The enhancement of the antiproliferative effects of cytostatic agents by glutamate antagonists was due to enhanced tumor cell death and to decreased cell division. Both (+)-dizocilpine and GYKI52466 were found to enhance the toxicity of cytostatic drugs at concentrations as low as 10 μM.

7. Mechanisms of the cytostatic effect of glutamate antagonists

The mechanisms involved in the cytostatic effects of glutamate antagonists will need to be worked out. Calcium appears to play a crucial role, since in the absence of calcium in the extracellular medium the antiproliferative effect of glutamate antagonists was markedly weakened. It is known that calcium can stimulate tumor growth [26,27], that calcium is necessary for cell division and survival [28–30], and that it regulates protein trafficking through the nuclear membrane [31]. Calcium also controls axon extension and pathfinding and influences cell migration [32–35]. It is known from developmental work that glutamate receptor/ion channels on embryonic neurons are permeable to calcium [36–38]. Thus, the interesting hypothesis arises whether calcium trafficking through the cytoplasmic membrane of tumor cells via glutamate receptor/ion channel complexes may be occurring to a much higher extent than it does in neurons, since the resting membrane potential in tumor cells is in the range of −30 to −50 mV [39,40]. In neurons, at such depolarized membrane potentials, the Mg^{2+} block of the NMDA receptor ion channel is relieved, and ion-permeability of both NMDA and AMPA glutamate receptor/ion channels increases compared to resting membrane potentials [41,42]. Blockade by glutamate antagonists of such an active ion-trafficking across the cytoplasmic membrane

may indeed be one mechanism to explain their antiproliferative action.

8. Future perspectives

Some new and major challenges are posed to researchers and the pharmaceutical industry by our studies. Determining whether glutamate antagonists exert similar cytostatic effects *in vivo* is one crucial issue to resolve. The molecular pathways that glutamate antagonists utilize to stop tumor cells from dividing and migrating will need to be identified. The electrophysiological and binding properties of glutamate receptor/ion channels on tumor cells will need to be investigated, and the glutamate receptor subunits expressed on tumor cells must be better characterized and sequenced. Based on these data, glutamate antagonists with the ability to block glutamate receptors/ion channels expressed on tumor cells will need to be developed or selected from the already existing drug libraries on the shelves of pharmaceutical companies, to allow for proof of concept trials in humans.

NMDA and AMPA antagonists enhanced tumoricidal effects of cytostatic drugs *in vitro* by inhibiting tumor cell proliferation and enhancing tumor cell death. These observations suggest that glutamate antagonists could add to the existing therapies of cancer.

Given the fact that relatively high concentrations of glutamate antagonists are necessary to block tumor cell proliferation, those compounds that do not penetrate the blood–brain barrier and cause no or little neurological side-effects appear to be the most favorable ones to be used in initial trials first.

References

[1] Watkins JC, Evans RH. Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 1981;21:165–204.

[2] Cavalheiro EA, Lehmann J, Turski L. Frontiers in excitatory amino acid research. New York: Alan R. Liss, 1988.

[3] Hayashi T. A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. Jpn J Pharmacol 1952;3:45–64.

[4] Hayashi T. Inhibition and excitation due to γ-aminobutyric acid in the central nervous system. Nature 1958;182:1076–7.

[5] Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers of the retina. Arch Ophthalmol 1957;58:193–201.

[6] Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 1969;164:719–21.

[7] Olney JW, Sharpe LG. Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Science 1969;166:386–8.

[8] Olney JW, Ho OL, Rhee V. Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp Brain Res 1971;14:61–76.

[9] Rothman SM. Synaptic activity mediates death of hypoxic neurons. Science 1983;220:536–7.

[10] Zipfel GL, Lee JM, Choi DW. Reducing calcium overload in the ischemic brain. N Engl J Med 1999;341:543–4.

[11] Turski L, Cavalheiro EA, Schoepp DD. Excitatory amino acids: ten years later. Amsterdam: IOS Press, 2001.

[12] Lee JM, Zipfel GJ, Choi DW. The changing landscape of ischaemic brain injury mechanisms. Nature 1999;399:A7–14.

[13] McNamara JO. Emerging insights into the genesis of epilepsy. Nature 1999;399:A15–22.

[14] Skerry TM, Genever PG. Glutamate signalling in non-neuronal tissues. Trends Pharmacol Sci 2001;22:174–81.

[15] Said SI, Berisha HI, Pakbaz H. Excitotoxicity in the lung: *N*-methyl-D-aspartate-induced, nitric oxide-dependent, pulmonary edema is attenuated by vasoactive intestinal peptide and by inhibitors of poly(ADP-ribose) polymerase. Proc Natl Acad Sci USA 1996;93:4688–92.

[16] Chenu C, Serre CM, Raynal C, Burt-Pichat B, Delmas PD. Glutamate receptors are expressed by bone cells and are involved in bone resorption. Bone 1998;22:295–9.

[17] Patton AJ, Genever PG, Birch MA, Suva LJ, Skerry TM. Expression of an *N*-methyl-D-aspartate-type receptor by human and rat osteoblasts and osteoclasts suggests a novel glutamate signaling pathway in bone. Bone 1998;22:645–9.

[18] Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. Science 1993;260:95–7.

[19] Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev 1979;3:79–83.

[20] Ikonomidou C, Bosch F, Miksa M, Bittigau P, Vöckler J, Dikranian K, Tenkova TI, Stefovska V, Turski L, Olney JW. Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. Science 1999;283:70–4.

[21] Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, Price MT, Stefovska V, Hoerster F, Tenkova T, Dikranian K, Olney JW. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science 2000;287:1056–60.

[22] Rzeski W, Turski L, Ikonomidou C. Glutamate antagonists limit tumor growth. Proc Natl Acad Sci USA 2001;98:6372–7.

[23] Peet NM, Grabowski PS, Laketić-Ljubojević I, Skerry TM. The glutamate receptor antagonist MK801 modulates bone resorption *in vitro* by a mechanism predominantly involving osteoclast differentiation. FASEB J 1999;13:2179–85.

[24] Laketić-Ljubojević I, Suva LJ, Maathuis FJM, Sanders D, Skerry TM. Functional characterization of *N*-methyl-D-aspartic acid-gated channels in bone cells. Bone 1999;25:631–7.

[25] Behar TN, Scott CA, Greene CJ, Wen X, Smith SV, Maric D, Liu QY, Colton CA, Barker JL. Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. J Neurosci 1999;19:4449–61.

[26] Meloni F, Brochieri A, Ballabio PC, Tua A, Grignani G, Grassi GG. Bombesin, calcium homeostasis and tumour growth. Monaldi Arch Chest Dis 1998;53:405–9.

[27] Celli A, Treves C, Nassi P, Stio M. Role of 1,25-dihydroxyvitamin D_3 and extracellular calcium in the regulation of proliferation in cultured SH-SY5Y human neuroblastoma cells. Neurochem Res 1999;24:691–8.

[28] Gleason EL, Spitzer NC. AMPA and NMDA receptors expressed by differentiating *Xenopus* spinal neurons. J Neurophysiol 1998;79:2986–98.

[29] Clapham DE. Calcium signaling. Cell 1995;80:259–68.

[30] Horner PJ, Gage FH. Regenerating the damaged central nervous system. Nature 2000;407:963–70.

[31] Stehno-Bittel L, Perez-Terzic C, Clapham DE. Diffusion across the nuclear envelope inhibited by depletion of the nuclear Ca^{2+} store. Science 1995;270:1835–8.

[32] Marks PW, Maxfield FR. Transient increases in cytosolic free calcium appear to be required for the migration of adherent human neutrophils. J Cell Biol 1990;110:43–52.

[33] Nakato K, Furuno T, Inagaki K, Teshima R, Terao T, Nakanishi M. Cytosolic and intranuclear calcium signals in rat basophilic leukemia

cells as revealed by a confocal fluorescence microscope. Eur J Biochem 1992;209:745–9.

[34] Lawson MA, Maxfield FR. Ca^{2+}- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. Nature 1995;377:75–9.

[35] Gomez TM, Spitzer NC. *In vivo* regulation of axon extension and pathfinding by growth-cone calcium transients. Nature 1999;397: 350–5.

[36] Gallo V, Pende M, Cherer S, Molne M, Wright P. Expression and regulation of kainate and AMPA receptors in uncommitted and committed neural progenitors. Neurochem Res 1995;20:549–60.

[37] Bardoul M, Levallois C, Konig N. Functional AMPA/kainate receptors in human embryonic and foetal central nervous system. J Chem Neuroanat 1998;14:79–85.

[38] Scherer SE, Gallo V. Expression and regulation of kainate and AMPA receptors in the rat neural tube. J Neurosci Res 1998;52:356–68.

[39] Iwata M, Komori S, Unno T, Minamoto N, Ohashi H. Modification of membrane currents in mouse neuroblastoma cells following infection with rabies virus. Br J Pharmacol 1999;126:1691–8.

[40] Sonnier H, Kolomytkin OV, Marino AA. Resting potential of excitable neuroblastoma cells in weak magnetic fields. Cell Mol Life Sci 2000;57:514–20.

[41] Burnashev N, Zhou Z, Neher E, Sakmann B. Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. J Physiol (Lond) 1995;485:403–18.

[42] Jonas P, Burnashev N. Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. Neuron 1995;5: 987–90.