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N-Methyl-D-Aspartate Receptor in Human Prostate Cancer

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Abstract. Expression of the N-methyl-D-aspartate receptor (NMDAr) and its involvement in cellular proliferation is well-known in tumors of neuronal tissue, such as glioma and neuroblastoma. We have investigated NMDAr expression in the normal, hyperplastic and neoplastic human prostate by immunohistochemistry. Low stromal NMDAr immunostaining was observed in 2 of 12 (17%) normal prostate specimens, but epithelial NMDAr staining was not seen. Of 18 benign prostatic hyperplasia (BPH) specimens, none had stromal NMDAr staining, but 2 had low and 1 had high epithelial NMDAr immunoreactivity. Moderate to high NMDAr immunostaining was observed in the stroma of 60 of 145 (41%) prostate cancer (PCa) specimens. Epithelial NMDAr staining was low in 26 (18%) and moderate to high in 36 (25%) of 145 PCa specimens. We have also examined the effects of the NMDAr antagonist memantine on the growth of ten human cancer cell lines: four prostate, two breast and four colon. The NMDAr antagonist memantine inhibited in-vitro growth of all ten cell lines, with half-maximal growth-inhibition at 5 to 20 μg/ml (23 to 92 μM) memantine. An NMDA agonist, L-cysteinesulfinic acid, stimulated cellular proliferation of all ten cell lines, with maximal growth-stimulation (30% to 75%, depending on the cell line) observed between doses of 33 to 66 μM. Our data provide evidence for the expression and activity of NMDAr in prostate cancer.

Key words: NMDA — Memantine — L-Cysteinesulfinic acid — Cancer — Prostate — Breast — Colon

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Introduction

Glutamate is the major excitatory neurotransmitter in the brain. It acts at cationic channels gated by three glutamate receptor subtypes: 1) N-methyl-D-aspartate (NMDA), 2) alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and 3) kainate. Glutamate also has metabotropic receptors, which are G protein-coupled and modify neuronal as well as glial excitability (Meldrum, 2000). The NMDA ligand-gated ion-channel is permeable to the cations Na^+, K^+ and, importantly, Ca^{2+}. Presence of the NMDA receptor (NMDAr) and its involvement in cellular proliferation is well-known in tumor cells derived from neuronal tissue, such as glioma and neuroblastoma (Yoshioka et al., 1996; North et al., 1997; Takhiro et al., 2001). However, not much is known about NMDAr expression and activity in other tumor types. We have found NMDAr expression in primary human prostate, breast and colon cancer specimens. Also, we find that the NMDAr antagonist memantine inhibits the growth of human cell lines from the three cancer types. Moreover, an NMDAr agonist stimulated the proliferation of these cell lines. Our data suggest the participation of NMDAr in the growth of the three solid malignancies studied.

Materials and Methods

Immunohistochemistry was done as previously described (Abdul & Hoosein, 2002) using anti-NMDAR1 antibody (4 μg in 300 μl PBS, rabbit polyclonal, Chemicon, Temecula, CA) and secondary anti-rabbit antibodies, conjugated to horseradish peroxidase. According to the manufacturer, the primary antibody is selective for the splice variants NR1-1a, NR1-1b, NR1-2a and NR1-2b. For human prostate multi-tissue, microarray slides were obtained from the Cooperative Prostate Cancer Tissue Resource (CPCTR) and the Cooperative Human Tissue Network (CHTN) under the tissue array program (TARP) of the National Cancer Institute (NCI, NIH, Bethesda, MD). For breast and colon tissue, microarray slides were obtained from the CHTN (TARP, NCI). Immunostaining levels were determined by visual scoring of the

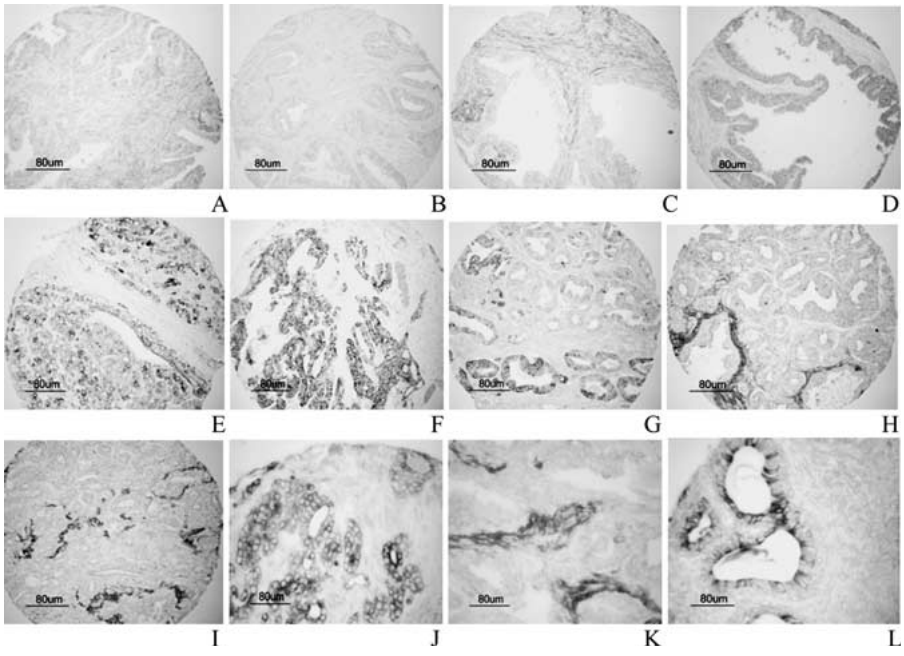

Fig. 1. Immunohistochemistry showing NMDA receptor staining in the normal human prostate (*A-C*), benign prostatic hyperplasia (BPH) (*D* and *L*) and prostate cancer (*E-K*) specimens.

brown product of the peroxidase substrate 3,3′-diaminobenzidine (DAB, Sigma Fast), in the absence of counterstain.

To study the effect of memantine on growth, ten cells lines (PC3, DU145, LNCaP and MDA-PCA-2B prostate cancer; MCF-7 and MDA-MB-231 breast cancer; LoVo, Colo320DM, LS174t and SW1116 colon cancer) were obtained from the American Type Culture Collection (ATCC). Cell lines were plated at 30,000 cells per well in 96-well plates, in serum-supplemented Leibovitz's-medium (L-medium). Two days later, cells were treated with channel blockers in serum-free L-medium. Cell numbers were determined 6 days later, using calcein AM (Molecular Probes, Eugene, OR). To study the effect of L-cysteinesulfinic acid (LCSA) on growth, cell lines were plated at 7,000 cells per well in 96-well plates in serum-supplemented L-medium. Next day, cells were changed to serum-free L-medium and 48 h later, cells were treated with LCSA in serum-free L-medium. After treatment for 72 h cell numbers were determined using calcein AM.

Graphpad (San Diego, CA) and Statsoft Statistica (Tulsa, OK) software were used for data analysis.

Results

Immunostaining with the anti-NMDAr antibody is shown in Fig. 1. Of 12 normal prostate specimens, 2 (17%) showed low levels of stromal NMDAr expression (Fig. 1*A* and *C*), but no significant epithelial staining was observed (Fig. 1*A-C*). Of 18 benign prostatic hyperplasia (BPH) specimens, none had stromal NMDAr, whereas 2 (11%) had low (Fig. 1*D*) and 1 (6%) had high (Fig. 1*L*) epithelial NMDAr expression. Of 145 prostate cancer (PCa) specimens, stromal NMDAr staining was low in 43 (30%), moderate in 38 (26%) and high in 22 (15%, Fig. 1*H*, *I* and *K*). In PCa specimens, epithelial NMDAr staining was low in 26 (18%), moderate in 24 (17%) and high in 12 (8%, Fig. 1*E-G* and *J*). 81 (56%) PCa specimens had either stromal or epithelial staining, whereas 42 (29%) had both stromal and epithelial NMDAr staining. Thus, it appears that both stromal and epithelial NMDAr expression is higher in prostate cancer compared to the normal prostate.

Tumor grade (Gleason score) of the 145 prostate cancer specimens was 5 in 7 (5%), 6 in 48 (33%), 7 in 75 (52%), 8 in 10 (7%) and 9 in 5 (3%) specimens. Thus, the vast majority (85%) of the prostate cancer specimens in this study had a Gleason score of 6 or 7. Tumor stage (TNM Staging) of the 145 prostate cancer specimens was 2a in 19 (13%), 2b in 76 (52%), 3a in 25 (17%) and 3b in 25 (17%). Thus, the majority of patients in this study had localized disease. Importantly, in the group of patients with a Gleason score of 5 or 6, there was a significant correlation between stromal NMDAr staining and tumor stage ($r = 0.32, p < 0.05, n = 55$), suggesting that an increase in stromal NMDAr staining could predict for a poor outcome.

All 10 normal breast specimens examined showed low to moderate staining in the stroma. Epithelial NMDAr expression, at moderate to high levels, was observed in 7 of 30 (23.3%) breast cancer specimens (*not shown*). No stromal or epithelial NMDAr expression was seen in 8 normal colon specimens. Of 45 colon cancer specimens examined, 8 (17.8%) had moderate to high levels of epithelial NMDAr expression (*not shown*).

The NMDAr antagonist memantine inhibited in-vitro growth of all ten cell lines studied (Fig. 2). Values for half-maximal inhibition varied from 5 to 20 μg/ml (23 to 92 μM). The NMDA agonist LCSA, stimulated cellular proliferation of all ten cell lines at doses between 5 to 50 μg/ml (Fig. 3). For most of the cell lines, maximal stimulation was observed at 5 to 10 μg/ml (33 to 66 μM). Maximal stimulation varied from 30% to 75%, depending on the cell line.

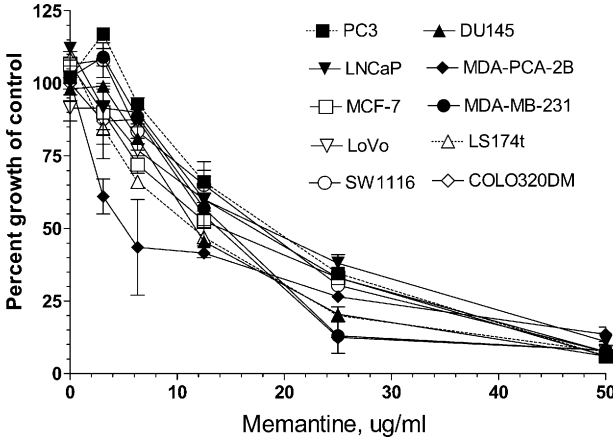

Fig. 2. Effect of memantine on the growth of human prostate (PC3, DU145, LNCaP, MDA-PCA-2B), breast (MCF-7, MDA-MB-231) and colon (LoVo, Colo320DM, LS174t and SW1116) cancer cell lines. Experiments were repeated three times. Results of a single experiment with triplicate assay points (mean ± SD) are shown.

Glutamate has been reported to promote the growth of A549 human lung cancer and TE671 human rhabdomyosarcoma/medulloblastoma cell lines (Rzeski, Turski & Ikonomidou, 2001).

Discussion

Our data provide evidence for the expression and activity of NMDA-gated ion channels in prostate as well as breast and colon cancer. We found an increased frequency and higher level of NMDAr expression in prostate cancer compared to the normal prostate (Fig. 1). NMDAr expression in the normal prostate has been previously reported (Gonzalez-Cadavid et al., 2000). NMDAr protein and NMDAR1 mRNA were detected in human and rat prostate by Western blot and reverse transcription polymerase chain reaction, respectively (Gonzalez-Cadavid et al., 2000).

At micromolar doses, memantine has been previously reported to inhibit the growth of A549 lung cancer and TE671 rhabdomyosarcoma/medulloblastoma cell lines (Rzeski, Turski & Ikonomidou, 2001). Two other NMDA antagonists MK 801 (dizocilpine) and ketamine also inhibited growth, the order of potency for growth-inhibition being memantine≥dizocilpine > > ketamine (Rzeski, Turski & Ikonomidou, 2001). The antiproliferative effects of the glutamate antagonists was Ca^{2+}-dependent and was attributed both to decreased cell-division as well as increased cell death. Memantine is a clinically well-tolerated, uncompetitive NMDA antagonist, currently used in the treatment of Parkinson's disease (Parsons, Danysz & Quack, 1999). Recently, memantine was shown to have activity in moderate to severe Alzheimer's disease (Tariot et al., 2004).

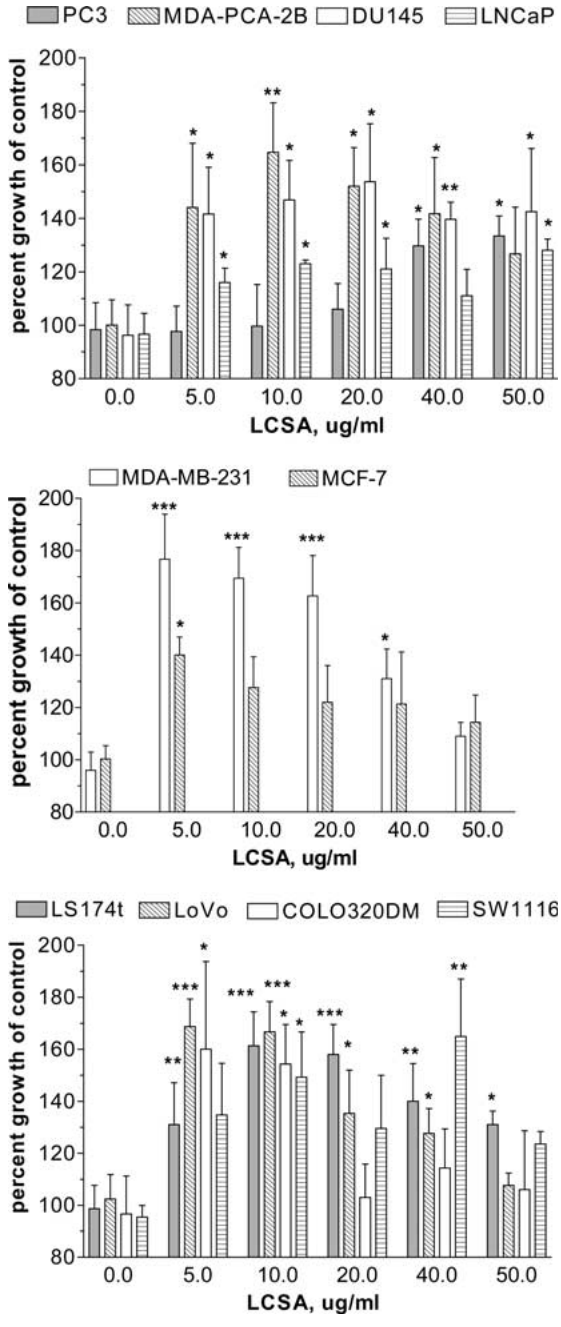

Fig. 3. Growth-stimulation by the NMDA agonist, L-cysteine sulfinic acid (LCSA). Experiments were repeated at least three times, with similar results. Results of a single experiment with triplicate assay points (mean ± SD) are shown. Students' *t*-test: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

The antiproliferative effect of MK 801, on colon (HT-29) and breast (T47D) human cancer cell lines has also been reported (Rzeski, Ikonomidou & Turski, 2002). In addition, MK 801 enhanced the growth-inhibitory effects of chemotherapeutic agents such as cyclophosphamide, cisplatin and vinblastine (Rzeski, Turski & Ikonomidou, 2001). These findings, taken together with our results showing growth

inhibition by memantine (Fig. 2) as well as growth-stimulation by LCSA (Fig. 3) of human prostate, breast and colon cancer cell lines, suggest that NMDAr is involved in the growth of several cancer types and is a good target for therapy.

Glutamate activity in the central nervous system (CNS) has been very well studied and has been implicated in several neurological and psychiatric disorders (Rzeski, Ikonomidou & Turski, 2002; Skerry & Genever, 2001). Glutamate antagonists have demonstrated anxiolytic, anticonvulsant, muscle relaxant, sedative and neuroprotective properties (Rzeski, Ikonomidou & Turski, 2002). Recently, glutamate signaling has been reported in non-neuronal tissues such as bone, pancreas and skin, raising the possibility of more widespread glutamate receptor activity (Skerry & Genever, 2001). In the developing mammalian brain, glutamate is known to have a trophic function (Rzeski, Ikonomidou & Turski, 2002). Our findings support the involvement of the NMDA-type glutamate receptor in the proliferation of human prostate, breast as well as colon cancer cells and warrant further examination of NMDAr as a therapeutic target and a prognostic indicator.

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