GluR2-Dependent Properties of AMPA Receptors Determine the Selective Vulnerability of Motor Neurons to Excitotoxicity

P. VAN DAMME,1 L. VAN DEN BOSCH,1 E. VAN HOUTTE,1 G. CALLEWAERT,2 AND W. ROBBERECHT1
Laboratory for 1Neurobiology and 2Physiology, University of Leuven, B-3000 Leuven, Belgium

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Van Damme, P., L. Van Den Bosch, E. Van Houtte, G. Callewaert, and W. Robberecht. GluR2-dependent properties of AMPA receptors determine the selective vulnerability of motor neurons to excitotoxicity. J Neurophysiol 88: 1279–1287, 2002; 10.1152/jn.00163.2002. AMPA receptor-mediated excitotoxicity has been implicated in the selective motor neuron loss in amyotrophic lateral sclerosis. In some culture models, motor neurons have been shown to be selectively vulnerable to AMPA receptor agonists due to Ca2+ influx through Ca2+-permeable AMPA receptors. Because the absence of GluR2 in AMPA receptors renders them highly permeable to Ca2+ ions, it has been hypothesized that the selective vulnerability of motor neurons is due to their relative deficiency in GluR2. However, conflicting evidence exists about the in vitro and in vivo expression of GluR2 in motor neurons, both at the mRNA and at the protein level. In this study, we quantified electrophysiological properties of AMPA receptors, known to be dependent on the relative abundance of GluR2: sensitivity to external polyamines, rectification index, and relative Ca2+ permeability. Cultured rat spinal cord motor neurons were compared with dorsal horn neurons (which are resistant to excitotoxicity) and with motor neurons that survived an excitotoxic insult. Motor neurons had a higher sensitivity to external polyamines, a lower rectification index, and a higher relative Ca2+ permeability ratio than dorsal horn neurons. These findings confirm that motor neurons are relatively deficient in GluR2. The AMPA receptor properties correlated well with each other and with the selective vulnerability of motor neurons because motor neurons surviving an excitotoxic event had similar characteristics as dorsal horn neurons. These data indicate that the relative abundance of GluR2 in functional AMPA receptors may be a major determinant of the selective vulnerability of motor neurons to excitotoxicity in vitro.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of upper and lower motor neurons. AMPA receptor-mediated excitotoxicity has been implicated in this selective motor neuron loss (Bar-Peled et al. 1999; Carriedo et al. 1995, 1996; Hugon et al. 1989; Ikonomidou et al. 1996; Rothstein 1996; Rothstein et al. 1993; Shaw and Ince 1997).

This selective toxicity seems to be dependent on Ca2+ influx through Ca2+-permeable AMPA receptors (Carriedo et al. 1995, 1996; Greig et al. 2000; Van Den Bosch et al. 2000). The Ca2+ permeability of the AMPA receptor is largely determined by the presence of the GluR2 subunit in the receptor complex. Receptors containing GluR2 have a very low relative Ca2+ permeability compared with GluR2-lacking receptor channels (Hollmann et al. 1991). The impermeability to Ca2+ is attributable to a positively charged arginine at position 586 (Q/R site) instead of a genetically encoded neutral glutamine (Burnashev et al. 1992; Hume et al. 1991). This arginine residue at the Q/R site is introduced by editing of the GluR2 pre-mRNA (Sommer et al. 1991), which is virtually complete. Three other functional properties of AMPA receptors (sensitivity to channel block by external polyamines, current rectification, and single-channel conductance) also depend on the presence of GluR2. GluR2-lacking channels are easily blocked by external polyamines (Brackley et al. 1993; Herlitze et al. 1993), display a strong inward rectification (Boulter et al. 1990; Hollmann et al. 1991; Verdoorn et al. 1991), and have a higher single channel conductance (Swanson et al. 1997). The inward rectification is due to the permeation of intracellular polyamines in the channel pore of GluR2-lacking receptors at positive potentials (Donevan and Rogawski 1995; Kamboj et al. 1995; Koh et al. 1995).

Although Ca2+ influx through Ca2+-permeable AMPA receptors appears to be critical for the selective motor neuron vulnerability, conflicting evidence exists about the relative expression of GluR2 in motor neurons, both at the mRNA (Greig et al. 2000; Takuma et al. 1999; Tölle et al. 1993; Tomiyama et al. 1996; Vandenbergh et al. 2000b; Virgo et al. 1996; Williams et al. 1997) and at the protein level (Bar-Peled et al. 1999; Del Cano et al. 1999; Morrison et al. 1998; Shaw et al. 1999). A critical role for GluR2 in the survival of motor neurons in vivo is suggested by the fact that transgenic mice overexpressing a GluR2 gene that encodes an asparagine at the Q/R site (yielding Ca2+-permeable AMPA receptors) develop a motor neuron disease later in life (Feldmeyer et al. 1999). On the contrary, GluR2 knock-out mice display no overt motor neuron disorder (Jia et al. 1996).

In this study, we used a co-culture system of either rat spinal motor neurons or dorsal horn neurons grown on a pre-established astroglial feeder layer to study the role of GluR2 in excitotoxic cell death. As previously shown (Van Den Bosch et al. 2000), almost half of the motor neurons are killed by short exposures to kainate (KA), whereas most dorsal horn neurons survive such a treatment. This selective motor neuron death can be prevented by antagonists of AMPA receptors, antagonists of Ca2+-permeable AMPA receptors, and by removal of extracellular Ca2+ (Van Den Bosch et al. 2000, 2002b). Using
the perforated-patch-clamp technique, we studied GluR2-dependent properties of AMPA receptors currents [sensitivity to the selective Ca\(^{2+}\)-permeable AMPA receptor antagonist 1-naphthyl acetyl spermine (NAS), rectification index and Ca\(^{2+}\) permeability] in both cell types and correlated these findings to KA-induced cell death. In contrast to GluR2 protein or mRNA detection, these properties relate only to the GluR2 content of functional AMPA receptors in the cell membrane.

**METHODS**

**Cell cultures**

Motor neurons were cultured as previously described (Vandenberge et al. 1998; Van Den Bosch et al. 2000), following procedures approved by the local ethical committee. In brief, ventral spinal cords were dissected from 14-day-old Wistar rat embryos in Hanks’ balanced salt solution (HBSS), cut in pieces of about 1 mm and digested for 15 min at 0.05% trypsin in HBSS at 37°C. After treatment with DNase, the tissue was further dissociated by trituration. A motor neuron-enriched neuronal population was purified from the ventral spinal cord by centrifugation on a 6.5% metrizamide cushion and was cultured on a glial feeder layer, which had been preestablished on 18-mm round glass coverslips coated with poly-l-ornithine and laminin. The culture medium consisted of L15 supplemented with sodium bicarbonate (0.2%), glucose (3.6 mg/ml), progesterone (20 nM), insulin (5 \(\mu\)g/ml), putrescine (0.1 mM), conalbumin (0.1 mg/ml), sodium selenite (30 nM), penicillin (100 IU/ml), streptomycin (100 \(\mu\)g/ml), and horse serum (2%). As previously described, most of the cells in culture are motor neurons as shown by immunostainings with the motor neuron marker peripherin (Van Damme et al. 2002). Dorsal horn neurons were dissociated from the dorsal spinal cord using the same protocol, except that the metrizamide gradient centrifugation was omitted.

Cultures were kept in a 7% CO\(_2\) humidified incubator at 37°C. Neurons were used for experiments between 7 and 9 days in culture.

**Toxicity experiments**

Motor neuron cultures after 8 days in culture were exposed to 300 \(\mu\)M KA for 30 min at 37°C in a modified Krebs solution [which contained (in mM) 122.3 NaCl, 5.9 KCl, 10 CaCl\(_2\), 1.2 MgCl\(_2\), 11.6 HEPES, and 11.5 glucose]. The Na\(^{+}\)-rich extracellular solution contained (in mM) 115 NaCl, 30 TEACl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 15.8 glucose, pH adjusted to 7.3 with NaOH. The Na\(^{+}\)-rich extracellular solution consisted of (in mM) 115 NaCl, 30 TEACl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 15.8 glucose, pH adjusted to 7.3 with NaOH. The Ca\(^{2+}\)-rich extracellular solution consisted of (in mM) 30 CaCl\(_2\), 30 TEACl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 15.8 glucose, pH adjusted to 7.3 with NaOH. The Na\(^{+}\)-rich extracellular solution contained (in mM) 105 NaCl, 30 TEACl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 15.8 glucose, pH adjusted to 7.3 with NaOH. The Ca\(^{2+}\)-rich extracellular solution consisted of (in mM) 30 CaCl\(_2\), 30 TEACl, 5.4 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 15.8 glucose, pH adjusted to 7.3 with NaOH. All experiments were carried out in the presence of 500 nM tetrodotoxin (TTX), 1 \(\mu\)M MK-801, and 100 \(\mu\)M Cd\(^{2+}\) to block voltage-gated Na\(^{+}\) channels, NMDA receptors, and voltage-operated Ca\(^{2+}\) channels, respectively. NAS was applied as a selective antagonist of GluR2-lacking AMPA receptors (Blaschke et al. 1993; Koike et al. 1997). Pentobarbital (PB), up to 100 \(\mu\)M, was used as a selective blocker of GluR2-containing AMPA receptors (Tavera et al. 1994; Yamakura et al. 1995).

The rectification of KA-induced currents was quantified using the following expression (Ozawa et al. 1991): Rectification index = \(\frac{I_{V0}/(40 - E_{rev})}{I_{V0}/(-60 - E_{rev})}\).

The Ca\(^{2+}\) permeability ratios, \(P_{Ca}/P_{Na}\), were determined from the reversal potentials obtained in a Na\(^{+}\)-rich \((V_{revNa})\) and a Ca\(^{2+}\)-rich solution \((V_{revCa})\) according to the equation (Geiger et al. 1995): \(P_{Ca}/P_{Na} = 0.25 \frac{a_{Ca}}{a_{Na}} \left[\frac{\exp(2(V_{revCa} - V_{revNa})/F/RT)}{\exp(V_{revCa} - V_{revNa})/F/RT}\right]\), where \(a_{Na}\) and \(a_{Ca}\) are the ion activities of Na\(^{+}\) and Ca\(^{2+}\) in the extracellular solution and \(F\), \(R\), and \(T\) have their conventional meaning. Activity coefficients were estimated by interpolation of tabulated values (0.75 for NaCl, 0.55 for CaCl\(_2\)).

**Materials and statistics**

Media and additives were obtained from Gibco BRL (Grand Island, NY); TTX was from Calbiochem (San Diego, CA). MK-801 was from Tocris Cookson (Bristol, UK). All other chemicals were from Sigma (St. Louis, MO). For dose-response curves, a logistic equation was used to fit and calculate EC\(_{50}\) and statistical difference was calculated by difference of slope analysis. Student’s t-tests were used to calculate significance of the GluR2-dependent properties between the different cell populations. Because the distributions were not always Gaussian, significance was confirmed by a nonparametric test (Wilcoxon).

**RESULTS**

Motor neurons with a high sensitivity to NAS are vulnerable to KA-induced cell death

As a first electrophysiological parameter of the relative abundance of GluR2 in functional AMPA receptors, sensitivity to NAS was studied. Application of 100 \(\mu\)M KA induced a large inward current at negative membrane potentials that could be blocked to a variable degree by NAS selective antagonist of GluR2-lacking AMPA receptors. Dose-response curves of NAS were generated by applying increasing doses of...
NAS during KA application. Motor neurons were significantly more sensitive to NAS compared with dorsal horn neurons (P = 0.001, Fig. 1A). The sensitivity to NAS was also determined in motor neurons that were exposed to 300 µM KA for 30 min. to induce cell death. Interestingly, motor neurons surviving such an excitotoxic insult were less sensitive to NAS to a level comparable to dorsal horn neurons. The calculated EC50 for NAS was 5.57 ± 0.52, 9.40 ± 0.96, and 8.71 ± 1.04 µM in motor neurons (n = 32), dorsal horn neurons (n = 23), and motor neurons surviving a KA exposure (n = 23), respectively. The mean inhibition of KA currents by 100 µM NAS amounted to 43 ± 3.4% (n = 56), 26 ± 2.2% (n = 46, P = 0.0001), and 27 ± 2.3% (n = 30, P = 0.001) in motor neurons, dorsal horn neurons and motor neurons surviving a toxic KA exposure, respectively. Pentobarbital up to a concentration of 100 µM has been reported to be a selective antagonist of GluR2-containing AMPA receptors (Tavera et al. 1994; Yamakura et al. 1995). As expected, dorsal horn neurons were more sensitive to pentobarbital at low concentrations than motor neurons (P = 0.001, Fig. 1B). Again, motor neurons surviving a short KA exposure behaved like dorsal horn neurons. The estimated EC50 for pentobarbital was 513 ± 65, 373 ± 87, and 289 ± 30 µM in motor neurons (n = 10), dorsal horn neurons (n = 10), and motor neurons surviving a KA exposure (n = 7), respectively.

Sensitivity to NAS appeared to divide motor neurons into two populations, cells with a high (Fig. 2A) and cells with a low (Fig. 2B) sensitivity to NAS. We therefore studied the distribution of inhibition of AMPA receptor currents by 100 µM NAS (Fig. 3A). Taking the maximum inhibition (51%) in motor neurons surviving a toxic KA exposure as a limit value, 37.5% (21 of 56 cells) of motor neurons displayed a high NAS sensitivity. On the contrary, almost no dorsal horn neurons with a high sensitivity to NAS were found (Fig. 3B, 3 of 36 = 8.3%). Motor neurons with a high sensitivity to NAS were selectively killed by KA application, as these cells were no longer encountered following a short KA exposure (Fig. 3C). If motor neurons were divided into cells with a low and high sensitivity to NAS, the dose-response curves of the resistant motor neuron population almost fully matched with the dorsal horn neurons and differed clearly from the vulnerable motor neuron population (Fig. 1C).

**Fig. 1.** Sensitivity of AMPA receptor currents to 1-naphthyl acetyl spermine (NAS) and pentobarbital (PB) in motor neurons and dorsal horn neurons. A: NAS dose-response curve in motor neurons (●) and dorsal horn neurons (○, ○, ○), and motor neurons surviving a kainate (KA) exposure (●, ●, ●). Inset: an example of inhibition of the KA current at −60 mV by increasing doses of NAS in a dorsal horn neuron. Each point represents the mean ± SE of data from 32 motor neurons, 13 dorsal horn neurons, and 19 motor neurons surviving a KA exposure. Motor neurons are significantly more sensitive to external NAS block of AMPA receptors (P = 0.001), whereas motor neurons that survived an excitotoxic insult had values comparable to dorsal horn neurons. By means of a logistic fit, EC50 were estimated to be 5.57 ± 0.52, 9.40 ± 0.96, and 8.71 ± 1.04 µM for motor neurons, dorsal horn neurons, and motor neurons surviving a KA exposure respectively. B: PB dose-response curve in motor neurons (●, ●), dorsal horn neurons (○, ○, ○), and motor neurons surviving a KA exposure (●, ●, ●). Inset: an example of inhibition of the KA-current at −60 mV by increasing doses of PB in a motor neuron. Each point represents the mean ± SE of data from 10 motor neurons, 10 dorsal horn neurons, and 7 motor neurons surviving a KA exposure. High concentrations of PB resulted in a nonselective block of AMPA receptors. At low concentrations however, motor neurons were significantly less sensitive to PB (P < 0.001) than dorsal horn neurons. Again motor neurons that were resistant to an excitotoxic insult behaved like dorsal horn neurons. Data were fit with a logistic equation. EC50 values were 513 ± 65, 373 ± 87, and 289 ± 30 µM for motor neurons, dorsal horn neurons, and motor neurons surviving a KA exposure, respectively. C: NAS dose-response curve in dorsal horn neurons and in subpopulations of motor neurons. Data from the same neurons as shown in A, but the motor neurons are divided in 2 subpopulations: cells with a high (more than 51%, ●, ●) and low (less than 51%, ○, ○) sensitivity to NAS. The dose-response curve of motor neurons with a low sensitivity almost fully matched with the trace of motor neurons surviving a toxic KA exposure and with the trace obtained from dorsal horn neurons. Each point represents the mean value ± SE from 11 motor neurons with a high sensitivity to NAS (●, ●), from 21 motor neurons with a low sensitivity to NAS (○, ○), from 19 motor neurons surviving a KA exposure (●, ●, ●), and from 13 dorsal horn neurons (○, ○, ○).
Motor neurons with a strong inward rectification are selectively killed by KA

As a second GluR2-dependent property of AMPA receptors, the current rectification was studied. Most motor neurons displayed a clear inward rectification, with a mean rectification index (Ozawa et al. 1991) of 0.78 ± 0.026 (n = 48). In Fig. 2, an example of an I-V relation with (Fig. 2C) and without (Fig. 2D) a clear inward rectification is shown. In the presence of 100 µM NAS, the inward rectification was completely lost, whereas 100 µM pentobarbital did not affect the rectification index (Table 1). These results support the idea that 100 µM NAS and 100 µM PB act as selective antagonists of GluR2-lacking and -containing AMPA receptors, respectively. The rectification index determined in dorsal horn neurons was significantly higher than the value obtained in motor neurons (0.94 ± 0.027, n = 34, P < 0.0001), but was close to the value in motor neurons surviving a toxic KA exposure (0.90 ± 0.018, n = 24). The rectification index in motor neurons surviving a toxic KA exposure was significantly higher than the rectification index in motor neurons (P = 0.002). As indicated in Fig. 4A, there was a good inverse correlation between the rectifica-
Motor neurons with a high relative $Ca^{2+}$ permeability are lost during KA application

The relative $Ca^{2+}$ permeability ratios $P_{Ca}/P_{Na}$ were calculated from the reversal potentials in a $Na^{+}$-rich and a $Ca^{2+}$-rich solution, both in motor neurons before and after a short KA exposure and in dorsal horn neurons. In Fig. 2, an example of a motor neuron with a high (Fig. 2E) and a low (Fig. 2F) $P_{Ca}/P_{Na}$ is shown. $P_{Ca}/P_{Na}$ correlated with sensitivity to NAS in motor neurons (Fig. 5A, $r = 0.83$, $P < 0.0001$, $n = 20$). The mean value of $P_{Ca}/P_{Na}$ in motor neurons was significantly higher compared with dorsal horn neurons ($0.80 \pm 0.14$ and $0.36 \pm 0.07$, $n = 20$ and $13$, respectively, $P = 0.025$). Motor neurons surviving an excitotoxic insult had a value of $0.41 \pm 0.07$ ($n = 14$, $P = 0.041$). In the dorsal horn neuron population and in motor neurons surviving a toxic KA exposure, no cells with a $P_{Ca}/P_{Na}$ value above 0.86 were found (Fig. 5, B and C).

In accordance with previous findings (Vandenbergh et al. 2000a), we also found higher current densities in motor neurons ($22.1 \pm 1.3$ pA/pF at $-80$ mV, $n = 22$) than in dorsal horn neurons ($13.5 \pm 1.6$ pA/pF, $n = 23$, $P = 0.0001$). Motor neurons surviving a short KA exposure had current densities similar to dorsal horn neurons ($12.2 \pm 0.6$ pA/pF, $n = 19$, $P < 0.0001$).

Taken together, motor neurons had a higher NAS sensitivity, lower rectification index, a higher $Ca^{2+}$ permeability, and a higher current density than dorsal horn neurons. Motor neurons with a high NAS sensitivity, a low rectification index, a high $Ca^{2+}$ permeability, and a high current density were selectively lost during a short KA exposure.

**DISCUSSION**

Motor neurons are particularly vulnerable to KA-induced cell death (Bar-Peled et al. 1999; Carriedo et al. 1995, 1996; Hugon et al. 1989; Ikonomidou et al. 1996; Rothstein et al. 1993). $Ca^{2+}$ influx through $Ca^{2+}$-permeable AMPA receptors appears to play a key role in this selective vulnerability of motor neurons. Mesulam et al. (1992) showed that motor neurons were more susceptible to KA than dorsal horn neurons. However, the mechanism of this selective vulnerability is not clear. In this study, we determined the relative $Ca^{2+}$ permeability in motor neurons and dorsal horn neurons.

**TABLE 1. Rectification index in motor neurons and dorsal horn neurons**

<table>
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<tr>
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<th>Motor Neurons</th>
<th>Dorsal Horn Neurons</th>
<th>Motor Neurons Surviving KA Exposure</th>
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<tr>
<td>Rectification index ($n$)</td>
<td>0.78 ± 0.03</td>
<td>0.94 ± 0.03</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>Rectification index in the presence of NAS ($n$)</td>
<td>48</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>Rectification index in the presence of PB ($n$)</td>
<td>0.79 ± 0.04</td>
<td>1.01 ± 0.03</td>
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The rectification index of kainate (KA)-induced currents was determined in motor neurons, dorsal horn neurons, and motor neurons surviving a short KA exposure. Motor neurons have a significantly lower rectification index than dorsal horn neurons ($P < 0.0001$) and than motor neurons surviving a short KA exposure ($P = 0.0029$). The influence of $100 \mu M$ pentobarbital (PB) and $100 \mu M 1$-naphthyl acetyl spermine (NAS) on the rectification index was also checked in motor neurons and dorsal horn neurons. Results are shown as means ± SE. $P$ values (in parentheses) in the table are relative to the value without NAS or PB.
FIG. 4. Relation between rectification index and NAS sensitivity in motor neurons (A), dorsal horn neurons (B), and motor neurons surviving an excitotoxic insult (C). A: the rectification index (for calculation of rectification index see METHODS) and the inhibition of KA currents by 100 μM NAS at −60 mV were determined in 38 motor neurons. A wide range of rectification indices was found, and there was an inverse correlation between the rectification index and NAS sensitivity (r = −0.697, P < 0.0001). - - -, the lowest value obtained in motor neurons surviving a toxic KA exposure (0.72). Of 38 motor neurons tested, 12 (31.6%) are predicted to be killed during an excitotoxic insult. B: relation between the rectification index and NAS sensitivity determined in 34 dorsal horn neurons. The fitted linear curve represents the correlation found in motor neurons (A). Only 4 of 34 (11.7%) dorsal horn neurons were situated below the cutoff value for excitotoxic death. C: relation between the rectification index and NAS sensitivity from 24 motor neurons surviving a toxic KA exposure. Motor neurons with a low rectification index and a high NAS sensitivity were no longer encountered.

FIG. 5. Relation between \( P_{\text{Ca}}/P_{\text{Na}} \) and NAS sensitivity. A: from the reversal potentials in a Na\(^{+}\)- and Ca\(^{2+}\)-rich solution the \( P_{\text{Ca}}/P_{\text{Na}} \) was calculated in 20 motor neurons (for calculation of \( P_{\text{Ca}}/P_{\text{Na}} \) and composition of Na\(^{+}\)- and Ca\(^{2+}\)-rich solution, see METHODS). A good correlation between \( P_{\text{Ca}}/P_{\text{Na}} \) and NAS sensitivity was found (r = 0.83, P < 0.0001). - - -, the maximal value obtained in motor neurons surviving a toxic KA exposure (0.86). Eight of 20 motor neurons (40%) had a \( P_{\text{Ca}}/P_{\text{Na}} \) above this value. B: determination of \( P_{\text{Ca}}/P_{\text{Na}} \) in 13 dorsal horn neurons revealed no cells with a high \( P_{\text{Ca}}/P_{\text{Na}} \). The fitted linear curve corresponds to the correlation found in motor neurons (A). C: relation of \( P_{\text{Ca}}/P_{\text{Na}} \) and NAS sensitivity in 14 motor neurons surviving a toxic KA exposure. No cells with a high \( P_{\text{Ca}}/P_{\text{Na}} \) were found. The maximal value obtained (0.86) was used as the cutoff value for prediction of cell death.
motor neurons (Carriedo et al. 1995, 1996; Greig et al. 2000; Van Den Bosch et al. 2000). AMPA receptors are assembled from GluR1–4 subunits, and their properties largely depend on the presence of the GluR2 subunit. AMPA receptors lacking GluR2 or containing the unedited GluR2 subunit are characterized by a high Ca\(^{2+}\) permeability, inward rectification, and sensitivity to polyamine block (Boulter et al. 1990; Brackley et al. 1993; Herlitze et al. 1993; Hollmann et al. 1991; Jonas and Burnashev 1995; Verdoorn et al. 1991). Motor neurons are therefore expected to have a low GluR2 expression or a low GluR2 editing. Because GluR2 appears to be fully edited in most cell types (Paschen et al. 1994; Puchalski et al. 1994), including motor neurons (Greig et al. 2000; Vandenberghe et al. 2000b), most attention has been focused on the level of GluR2 expression. However, conflicting evidence exists about the relative expression of GluR2 in motor neurons, both at the mRNA (Greig et al. 2000; Tölle et al. 1993; Tomiyama et al. 1996; Vandenberghe et al. 2000b; Virgo et al. 1996; Williams et al. 1997) and at the protein level (Bar-Peled et al. 1999; Del Cano et al. 1999; Morrison et al. 1998; Shaw et al. 1999).

Rather than determining the relative abundance of GluR2, we have focused in this study on properties of AMPA receptors, which are known to be dependent on the relative abundance of the GluR2 protein. These properties relate only to the GluR2 content in functional AMPA receptors in the cell membrane. The three different properties were concordant in all experiments, suggesting that they reliably reflect the GluR2 content. These properties were studied in motor neurons (which are vulnerable to excitotoxicity), dorsal horn neurons (which are resistant to excitotoxicity), and in motor neurons that survived a toxic KA exposure. To avoid washout of intracellular polyamines, which are responsible for the inward rectification of GluR2-lacking AMPA receptor, the gramicidin perforated-patch-clamp technique was used.

We found that motor neurons had a significantly higher sensitivity to AMPA receptor block by NAS, a selective antagonist of GluR2-lacking AMPA receptors (Blaschke et al. 1993; Koike et al. 1997), compared with dorsal horn neurons. Inversely, dorsal horn neurons displayed a larger inhibition of AMPA receptor currents by low concentrations of PB than motor neurons. PB in concentrations up to 100 \(\mu\)M is believed to be a selective antagonist of GluR2-containing AMPA receptors (Taverna et al. 1994; Yamakura et al. 1995). Interestingly, the nonvulnerable subpopulation of motor neurons, which was defined as the population surviving a short KA exposure, displayed a similar sensitivity to NAS and PB as dorsal horn neurons. The fact that no motor neurons with a high sensitivity to NAS were encountered in the cells that survived a KA exposure suggests that these cells were selectively killed by KA. The similarity between the proportion of motor neuron death and the proportion of motor neurons with a high NAS sensitivity makes it less likely that major changes in the GluR2 content during the KA exposure (e.g., by downregulation of GluR2 or internalization of GluR2-containing receptors) are involved.

This idea was further supported by the analysis of rectification indices. As previously shown (Van Damme et al. 2002), application of KA gives rise to an inhibition of TEA\(^{+}\)-sensitive voltage-gated K\(^{+}\) channels. This inhibition leads to an apparent inward rectification of the KA-induced current. Reliable measurements of the KA-induced current are obtained in the presence of 30 mM external TEA\(^{+}\) because KA has no additional inhibitory effect on K\(^{+}\) channels under these circumstances. The mean value of rectification index in response to KA application was 0.78 ± 0.03, a value close to the value previously reported in motor neurons of rat spinal cord slices (Abdrachmanova et al. 2000) but was significantly lower than the value obtained in dorsal horn neurons (0.94 ± 0.03). There was a good correlation between the rectification index and NAS sensitivity and motor neurons with a low rectification index were selectively lost during a short KA exposure.

Similar data were obtained by comparing \(P_{\text{Ca}}/P_{\text{Na}}\) between the different cell groups. The mean value of \(P_{\text{Ca}}/P_{\text{Na}}\) obtained in motor neurons amounted to 0.80 ± 0.15. This value is higher than the \(P_{\text{Ca}}/P_{\text{Na}}\) value of about 0.4 found in cultured rat spinal cord neurons by others (Greig et al. 2000; Vandenberghe et al. 2000b). The fact that we used the perforated-patch-clamp technique whereas the two other groups used the whole cell configuration most likely does not contribute to this difference because we found a similar high \(P_{\text{Ca}}/P_{\text{Na}}\) value using the whole cell configuration (data not shown). Differences in culture conditions might explain this discrepancy. Alternatively, strain differences can be involved as some investigators used Holtzman rats, whereas we used Wistar rats. For mice, it has been shown that different strains can differ substantially in their vulnerability to excitotoxicity (Schauwecker and Steward 1997; Shuttleworth and Connor 2001). The value of \(P_{\text{Ca}}/P_{\text{Na}}\) obtained in dorsal horn neurons amounted to 0.36 ± 0.07 and was lower than the values found in previous studies (Goldstein et al. 1995; Vandenberghe et al. 2000b). Motor neurons in this study thus have a much higher relative Ca\(^{2+}\) permeability ratio compared with dorsal horn neurons. \(P_{\text{Ca}}/P_{\text{Na}}\) values correlated well with NAS sensitivity and consequently motor neurons with the highest Ca\(^{2+}\) permeability were selectively killed during a toxic KA exposure.

As has been reported previously (Vandenberghe et al. 2000a), we also found higher current densities in motor neurons compared with dorsal horn neurons. Furthermore, motor neurons with the highest current amplitudes were selectively lost during a short KA exposure. The higher current amplitudes in motor neurons is compatible with a relatively low expression of GluR2 because GluR2-lacking AMPA receptors are known to have a higher single-channel conductance (Swanson et al. 1997).

In short, the studied properties of AMPA receptors, all thought to be dependent on the relative abundance of GluR2, correlated well with each other and with KA-induced motor neuron death.

Using the limit values in the surviving motor neuron population (51% inhibition of AMPA receptor currents by 100 \(\mu\)M of NAS, a rectification index of 0.72 and a \(P_{\text{Ca}}/P_{\text{Na}}\) of 0.86), we estimated the proportion of vulnerable motor neurons between 31.6 and 40%. As previously demonstrated in our culture model (Van Den Bosch et al. 2000), 46 ± 2% \((n = 30)\) of motor neurons are killed during a short KA exposure, whereas only 7.3 ± 2% \((n = 5)\) of dorsal horn neurons are lost during a KA exposure. Thus our estimate of vulnerable motor neurons correlates well with the proportion of motor neuron death during a short KA exposure (Table 2). Using Co\(^{2+}\) staining as a histochemical marker for the presence of Ca\(^{2+}\)-permeable AMPA receptor, about 55% of motor neurons are marked as positive. If the number of Co\(^{2+}\) positive cells that are found in...
Summary of data obtained from motor neurons and dorsal horn neurons. Motor neurons were known to factors that contribute to the selective vulnerability of motor neurons. Previous studies have shown that the selective vulnerability of motor neurons in vivo is determined by a transgenic mice model with SOD1 mice (Beers et al. 2001). Increased Ca²⁺ buffering capacity (Alexianu et al. 1994; Ince et al. 1993; Lips and Keller 1998, 1999; Palecek et al. 1999; Vanselow and Keller 2000), which might render them more susceptible to Ca²⁺-mediated cell death. Furthermore, overexpression of the Ca²⁺-binding protein parvalbumin was shown to diminish excitotoxic motor neuron death (Van Den Bosch et al. 2001).

Evidence for the importance of GluR2 in motor neuron vulnerability in vivo came from a transgenic mice model with SOD1 mice. A single amino acid determines the subunit-specific spider toxin block of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor channels. In vitro kainate injury to spinal neurons is Ca²⁺-dependent. AMPA receptor channels are involved in the selective vulnerability of motor neurons to excitotoxicity (Carriedo et al. 2000).

Evidence for the importance of GluR2 in motor neuron viability in vivo came from a transgenic mice model with overexpression of a GluR2 gene that encodes an asparagine (GluR2-N) at the Q/R site (Feldmeyer et al. 1999). AMPA receptor channels incorporating GluR2-N are permeable to Ca²⁺ (Burnashev et al. 1992), and these mice were shown to develop a motor neuron degeneration later in life. However, transgenic mice that lack the GluR2 subunit, do not suffer from a motor neuron disease (Jia et al. 1996). This might be due to some adaptation during development to limit excessive AMPA receptor stimulation, such as a long latency for recovery from the desensitized state (Harvey et al. 2001). Further research is necessary to clarify the role of GluR2-lacking AMPA receptors in the selective vulnerability of motor neurons in vivo.

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### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Motor Neurons, %</th>
<th>Dorsal Horn Neurons, %</th>
</tr>
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<tbody>
<tr>
<td>CA⁻⁻⁺⁺-induced cell death</td>
<td>46 ± 2</td>
<td>7.3 ± 4.1</td>
</tr>
<tr>
<td>Ca²⁺-positivity</td>
<td>55.1 ± 1.5</td>
<td>8.2 ± 2.3</td>
</tr>
<tr>
<td>NAS sensitivity &gt;51%</td>
<td>37.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Rectification index &lt;0.72</td>
<td>31.6</td>
<td>11.8</td>
</tr>
<tr>
<td>P&lt;0.86</td>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

Summary of relation between cell death data and parameters of GluR2-lacking AMPA receptors

**References**


ROLE OF GLUR2 IN EXCITOTOXIC MOTOR NEURON DEATH


