Removal of NMDA Receptor Mg$^{2+}$ Block Extends the Action of NT-3 on Synaptic Transmission in Neonatal Rat Motoneurons

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Arvanian, Victor L. and Lorne M. Mendell. Removal of NMDA receptor Mg$^{2+}$ block extends the action of NT-3 on synaptic transmission in neonatal rat motoneurons. J Neurophysiol 86: 123–129, 2001. NT-3 has previously been reported to enhance AMPA/kainate receptor-mediated synaptic responses in motoneurons via an effect on the N-methyl-D-aspartate (NMDA) receptor. To investigate neurotrophin-3 (NT-3) action further, we measured the NMDA receptor (NMDAR)-mediated synaptic response directly by intracellular recording in motoneurons after blocking AMPA/kainate, GABA$_A$, GABA$_B$ and glycine receptor-mediated responses pharmacologically. Two pathways were stimulated, the segmental dorsal root (DR) and the descending ventrolateral fasciculus (VLF). The DR-evoked NMDAR-mediated response in motoneurons of rats younger than 1 wk has two components, the initial one of which is generated monosynaptically. NT-3 strongly potentiated both NMDA components in a rapidly reversible manner. No NMDAR-mediated responses were present at VLF connections and at DR connections in older (1- to 2-wk-old) neonates. Bath-applied NT-3–induced potentiation of the AMPA/kainate receptor-mediated response occurred only at connections that exhibit a synaptic NMDA receptor-mediated response. Reducing Mg$^{2+}$ concentration in the bathing solution restored the NMDAR-mediated response elicited by DR stimulation in older neonates and by VLF throughout the neonatal period (0–2 wk). In low-Mg$^{2+}$, NT-3 enhanced AMPA/kainate receptor-mediated responses elicited by inputs normally not influenced by NT-3. Thus a major reason for the loss of NT-3 action on AMPA/kainate synaptic responses is the reduced activity of the NMDA receptor due to developing Mg$^{2+}$ block of NMDA receptor-channel complex as the animal matures, and both can be re-established by reducing Mg$^{2+}$ concentration in fluid bathing the spinal cord.

INTRODUCTION

Studies of the physiological effects of neurotrophins in neonatal rat spinal cord have shown that neurotrophin-3 (NT-3), but not nerve growth factor (NGF), administered to isolated spinal cord acutely increases the amplitude of the fast monosynaptic AMPA/kainate-mediated excitatory postsynaptic potential (EPSP) evoked by electrical stimulation of dorsal root (DR) (Arvanov et al. 2000). This effect is age-specific in that NT-3 action on the DR-EPSP is restricted to very young (<1 wk old) animals. It is also synapse-specific in that NT-3 did not affect the AMPA/kainate-mediated EPSPs produced by activation of the descending ventrolateral funiculus (VLF) fibers in the same motoneurons whose AMPA/kainate receptor-mediated EPSPs from the DR were strongly facilitated. The action of NT-3 on the DR-EPSP was prevented by K-252a, an inhibitor of receptor tyrosine kinases (Knusel and Hefti 1992), suggesting the involvement of the trk family of receptor tyrosine kinases. Blockade of the N-methyl-D-aspartate (NMDA) receptor by D-2-amino-5-phosphovaleric acid (d-APV) prior to NT-3 administration also prevented its action, indicating that activation of NMDA receptors is required to initiate the NT-3-induced increase of AMPA/kainate receptor-mediated responses. MK-801 administered inside the motoneuron had an effect similar to bath-applied APV, suggesting that NMDA receptors in the motoneuron are crucial for the effect of NT-3 to be expressed.

Because trkC receptors for NT-3 are expressed on Ia afferents (McMahon et al. 1994) and α motoneurons (Johnson et al. 1996) throughout the life span of rats, we have hypothesized that the age-related changes in NT-3 action are likely to reflect changes in NMDA receptors. These undergo modification during the neonatal developmental period (Kalb et al. 1992) (see DISCUSSION). Specifically, we were drawn to the possibility that NMDA receptors in motoneurons become more susceptible to Mg$^{2+}$ block as the animal matures and that this might be the cause of the inability of NT-3 to affect synaptic transmission. To check this hypothesis, we studied the NMDA-mediated synaptic responses evoked by DR and VLF stimulation in animals in the first (1 WO) and second (2 WO) postnatal weeks. To accomplish this, we blocked the action of other known receptors pharmacologically by treating the isolated spinal cord with a “cocktail” made up of CNQX (to block AMPA/kainate receptors), strychnine (to block glycine receptors), bicuculline (to block GABA$_A$ receptors), and CGP35348 (to block GABA$_B$ receptors). Studies under these conditions revealed that the NMDA receptor (NMDAR)-mediated responses were enhanced by NT-3. Furthermore, changes in NMDAR-mediated responses were developmentally regulated, and changes in them paralleled the sensitivity of AMPA/kainate receptor-mediated responses to NT-3 at the same connections. Finally, elevating the sensitivity of the NMDA receptor by reducing Mg$^{2+}$ concentration in the bath increased the response of both the NMDA receptor and the associated AMPA/kainate receptors (studied in the absence of the blockers) to NT-3. Thus these experiments confirmed the relationship between NMDAR function and sensitivity to NT-3 and this suggests strategies for extending the effects of NT-3 in modulating synaptic transmission in the spinal cord.
A preliminary account has been presented in abstract form (Arvanov and Mendell 2000).

**METHODS**

These experiments were carried out in spinal cords removed from neonatal male Sprague-Dawley rats using methods previously described in detail (Arvanov et al. 2000; Fulton and Walton 1986; Seebach and Mendell 1996; Seebach et al. 1999). Two age groups were used: 1–5 days old (1WO) and 9–15 days old (2WO). After removal of the spinal cord from the animal, a section spanning segments from approximately T1 to S3 was placed in a chamber filled with 3 M potassium acetate (Sylgard)-coated surface in the recording chamber. The Ld dorsal and ventral roots as well as the cut VL (dissected from the spinal cord at T12) (Pinco and Lev-Tov 1994) were placed in suction electrodes with silver-silver chloride internal wires for stimulation. The preparation was then allowed to equilibrate slowly to 30°C over a period of 1–1.5 h and the experiment was carried out at 30°C.

Intracellular recordings (microelectrodes 70–110 MΩ filled with 3 M potassium acetate) were made in lumbar spinal motoneurons in the L5 segment that were identified by their antidromic response to VR stimulation. Initially, the synaptic response to 10 stimuli of 50-μs duration delivered separately to DR and VLF was averaged (pClamp 8, Axon Instruments). Stimulation rate was 0.05 or 0.1 Hz for experiments in which the fast monosynaptic EPSP was studied (Fig. 4), but only 0.01 Hz when NMDAR-mediated responses were studied (Fig. 1). Five-minute breaks were interspersed between groups of 10 stimuli in all experiments. To record the NMDAR-mediated synaptic responses, the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxalene-2,3-dione (CNQX, 10 μM), the GABA<sub>B</sub> receptor antagonist bicuculline (5 μM), the GABA<sub>α</sub> receptor antagonist CGP 35348 (10 μM) (Bertrand and Cazalets 1999; Peshori et al. 1998) and the glycine receptor antagonist strychnine (5 μM) were included in the perfusate to isolate NMDAR-mediated responses pharmacologically. All drugs were added to the perfusion solution. The neurotrophins NT-3 and NGF were administered at a concentration of 0.2 μg/ml. In some experiments, saline with reduced concentrations of Mg<sup>2+</sup> or increased concentration of Ca<sup>2+</sup> was substituted for the artificial bathing solution described in the preceding text. In these cases, corresponding equiosmolar changes in Na<sup>+</sup> concentration were made.

The peak of the monosynaptic component was detected most clearly at low stimulus intensity below the levels required to evoke the later components. A cursor was placed at this peak and used to measure the amplitude of the EPSP in the single sweep determined to contain the maximum monosynaptic response (see Figs. 1A1 and 3A). Motoneuron input resistance was estimated by passing current pulses (100 ms) through the intracellular recording electrode as described previously (Arvanov et al. 2000; Fulton and Walton 1986).

The results are presented as means ± SE. Each cell in the present data series was from a different spinal cord. t-tests were used to determine the significance of the differences. Bonferroni’s correction was used as required to adjust for multiple comparisons.

**RESULTS**

**NMDAR-mediated synaptic responses in motoneurons**

To study the NMDAR-mediated response in isolation, all other known inputs to the motoneuron were blocked pharmacologically, and synaptic responses evoked by stimulation of DR and VLF were studied as a function of stimulus intensity. In the presence of AMPA/kainate-, GABA<sub>α</sub>-GABA<sub>β</sub>- and glycine-receptor antagonists, the fast monosynaptic component of DR-EPSP was blocked. Under these conditions, low-intensity DR stimulation evoked a slightly slower response whose latency (6.4 ± 0.5 ms compared with 5.5 ± 0.4 ms in the same cells; n = 37; P > 0.05), threshold (86 ± 8 vs. 68 ± 6 μA; P > 0.05), and sensitivity to high-frequency stimulation (both followed repetitive stimulation only at frequencies of 0.05–0.1 Hz or lower) were similar to those of the initial component observed in the absence of the blockers. This response was completely blocked by APV, indicating that it was mediated by NMDA receptors. We conclude that this NMDA component was also monosynaptically driven (see also Pinco and Lev-Tov 1994) and refer to it as the NMDA-m response. A further increase of stimulus intensity (≥500 μA) and/or duration (≥500 μs) had no effect on maximum amplitude or latency of NMDA-m response (Fig. 1A1; see also Fig. 3A).

Once the NMDA-m component reached maximum amplitude, a further increase in stimulus intensity of 5–30 μA evoked a much later APV-sensitive component (150- to 250-ms latency at the peak), referred to as NMDA-l. The amplitude of NMDA-l increased to some intermediate value of DR and VLF stimulation described in the preceding text. In these cases, corresponding equiosmolar changes in Na<sup>+</sup> concentration were made.
and then jumped in an all-or-none fashion to a maximum value suggestive of a region of negative resistance in the motoneuron (Fig. 1A; n = 19). With further increase of stimulus intensity, the latency of NMDA-l decreased (see Fig. 3). However, this decrease in latency had no effect on the peak amplitude of the NMDA-m component (see Fig. 3A). NMDA-l could follow stimulus rates of only 0.01–0.02 Hz, considerably lower than those seen for the NMDA-m component (see preceding text). In this study, we did not investigate the properties of this complex presumably polysynaptic NMDA-l component further by subjecting the motoneuron to voltage clamp. Its polysynaptic linkage makes interpretation of changes after neurotrophin administration equivocal, and so the evaluation of the action of these agents is restricted to their effect on NMDA-m response.

Additional experiments (n = 6) were carried out with the NMDA antagonist MK-801 in the recording electrode (Arvanov et al. 2000). In these experiments, the antagonist cocktail (without APV) was introduced into the bathing solution ~0.5 h before penetration of the motoneuron. In all six cells recorded with the electrode containing MK-801, we were able to record the first DR-evoked NMDAR-mediated response that disappeared within 10–20 min (Fig. 2), suggesting that the NMDA receptors that mediate these responses are located in the motoneuron membrane. Note, in the control recordings performed with electrodes filled with 3 M potassium acetate without MK-801, the DR-evoked NMDA receptor-mediated synaptic responses persisted with no decay for ≥1 h in the presence of the antagonist cocktail (without APV) in ACSF (n = 37; see Fig. 3).

VLF stimulation elicited no NMDAR-mediated responses on motoneurons in 1 WO animals in contrast to such responses evoked in the same motoneuron by DR-stimulation (n = 17; Figs. 1 and 4). Similarly, only very small or in many cases no NMDAR-mediated responses (mean = 0.4 ± 0.1 mV, n = 17, Figs. 1 and 4) were observed at DR-synapses on motoneurons in 2 WO animals. VLF stimulation also failed to induce the NMDA response in 2 WO animals (n = 14, Table 1).

**FIG. 2. Selective blockade of motoneuron NMDA-receptor by MK-801 (500 μM) included in the recording electrode. The entire DR-evoked response was abolished 20 min after penetration of motoneuron, indicating that it was NMDAR-mediated. In these experiments, the non-NMDAR antagonist cocktail was introduced into the bathing solution ~0.5 h before penetration of the motoneuron.**

**FIG. 3. Neurotrophin-3 (NT-3), but not but not nerve growth factor (NGF) facilitates the DR-evoked NMDA-m response in a motoneuron from a 1 WO rat. A: NMDA responses, evoked in the presence of non-NMDAR antagonist cocktail by the DR stimulation at various intensities, 1: 125 μA (intensity at which maximum amplitude of NMDA-m component was noted), 2: 200 μA, and 3: 500 μA, respectively. At higher stimulus intensity, the NMDA-l component became evident and its latency decreased with further increased stimulus intensity. B: NMDA-responses evoked in the same cell by 200-μA stimulus (suprathreshold for maximum NMDA-m response) at 0.01 Hz in the presence of non-NMDAR antagonist cocktail (control; 1); bath-applied NGF (0.2 μg/ml) did not alter NMDA-response (2); 30 min washout of NGF (3); in same motoneuron NT-3 (0.2 μg/ml) enhanced peak amplitude of NMDA-m response (peak amplitude of NMDA-m response remained enhanced while NT-3 was present in the bath; 30 min) (4); 20 min after washout of NT-3 the amplitude of the response had decayed close to the baseline level (5). Note that NT-3-induced increase in the peak amplitude of NMDA-m component was accompanied by a shift of the NMDA-l component to the left. This NT-3 induced shift of NMDA-l component was comparable to that induced by the higher-intensity (500 μA) stimulus in the absence of NT-3 in the same cell (see A), and neither altered the time course of the NMDA-m component.**
control levels within 20–30 min of washout of the NT-3 (Fig. 3). In the same motoneurons NGF had no effect on these synaptic responses (Fig. 3, mean = 3.1 ± 1.04 mV in control vs. 3.32 ± 1 in NGF, n = 5, P = 0.98).

Extending NMDA receptor function

As mentioned in the preceding text, pharmacological blockade of AMPA/kainate, GABA<sub>A</sub>, GABA<sub>B</sub>, and glycine receptors revealed no NMDAR-mediated responses at VLFC connections on motoneurons in 1 WO animals or on DR-synapses in 2 WO animals (Fig. 1 and 4). Activation of NMDA receptors depends on extracellular Mg<sup>2+</sup> concentration because of the voltage-dependent Mg<sup>2+</sup> block of these receptors (Ault et al. 1980; Nowak et al. 1984). We investigated whether the absence of NMDAR-mediated synaptic responses as a function of stimulus source (DR, VLFC) or age (1 or 2 WO) was due to a systematically higher sensitivity of motoneuron NMDA receptors associated with these inputs to Mg<sup>2+</sup>. Reducing the level of Mg<sup>2+</sup> in the bathing solution to 1 μM increased the peak amplitude of the existing DR-activated NMDA-m response in 1 WO animals to 6.2 ± 1.2 mV from 3.2 ± 0.4 mV measured in control solution with 2 mM Mg<sup>2+</sup> (Table 1, n = 5). Notably, NMDAR-mediated responses elicited in 2 WO animals by DR stimulation (n = 6; Fig. 4, Table 1) and VLFC stimulation (n = 9; Fig. 4, Table 1) became evident, increasing significantly from the negligible values in 2 mM Mg<sup>2+</sup>. Input resistance of the cells in low Mg<sup>2+</sup> did not change significantly indicating that the larger NMDAergic EPSP was not due to changes in passive properties of the motoneurons.

### NT-3 effects are extended in parallel with NMDA receptor function

In solutions containing low Mg<sup>2+</sup> (1 μM) the ability of NT-3 to affect synaptic responses was extended to new inputs (VLFC) and at both afferent and descending inputs beyond the usual postnatal 1-wk limit. Under these conditions NT-3 enhanced the amplitude of the “uncovered” NMDAR mediated responses [52.7 ± 10% VLFC 1WO (n = 4), 62.4 ± 11.7% DR 2WO (n = 6), 6.5 ± 12.4% VLFC 2WO (n = 5)], similar to the increase observed for DR 1WO NMDA responses in low 1 μM Mg<sup>2+</sup> (72.7 ± 9.1%, n = 5) or standard 2 mM Mg<sup>2+</sup> (59.1 ± 10.2%, n = 9) solutions (Fig. 4 and Table 1). NT-3 also significantly facilitated AMPA/kainate receptor-mediated EPSPs in cells displaying novel DR or VLFC activated NMDAR-mediated responses (Fig. 4 and Table 1).

We conclude that a major reason for the loss of NT-3 action in normal ASCF is the reduced activity of the NMDA receptor as the animal matures. Lowering extracellular Mg<sup>2+</sup> results in the reappearance of NMDA transmission and this extends the action of NT-3 on the AMPA-kainate response.

### Effects of increased levels of Ca<sup>2+</sup>

Reducing extracellular Mg<sup>2+</sup> (1 μM Mg/2.5 mM Ca) or increasing Ca<sup>2+</sup> (2 mM Mg/4 mM Ca) were compared at each age (1 WO, 0–1 week postnatal; 2 WO, 1–2 week postnatal) and with each stimulus (dorsal root (DR) and ventrolateral funiculus (VLFC)) to data obtained in standard Mg<sup>2+</sup>/Ca<sup>2+</sup> (2 mM Mg/2.5 mM Ca) solutions. The data were compared using t-tests corrected for multiple comparisons with the Bonferroni correction. Two levels of significance are denoted: *P = 0.002, equivalent to uncorrected value of P = 0.05; and **P = 0.0003, equivalent to uncorrected value of P = 0.01. Note the parallel increase in the N-methyl-D-aspartate receptor (NMDAR)-mediated response and in the effects of neurotrophin-3 (NT-3) on both the NMDAR- and the AMPA/kainate receptor-mediated responses in low Mg<sup>2+</sup> but not in high Ca<sup>2+</sup>. Values are means ± SE. Parentheses enclose n values.
FIG. 4. Decrease in extracellular Mg$^{2+}$ unmasks NMDA responses and extends facilitatory action of NT-3 to AMPA/kainate receptor-mediated responses evoked by stimulation of VLF and DR in 2 WO animals. Representative traces (left) demonstrate that in normal ACSF NT-3 enhanced DR-evoked EPSP (top row), but not VLF-evoked EPSP in the same motoneuron from 1 WO rat (middle row), nor DR-EPSP in 2WO rat (bottom row). After administration of non-NMDA antagonists (see Fig. 1) stimulation of DR evoked NMDA synaptic response in 1 WO animal (top middle). In the same cell stimulation of VLF failed to evoke a NMDA response (middle), nor was an NMDA response present at DR-connections in 2 WO animals (bottom middle). Decreasing extracellular Mg$^{2+}$ from 2 mM to 1 $\mu$M in ACSF containing the non-NMDA antagonists enhanced DR-evoked NMDA responses in 1 WO animal (top middle). Similar NMDA-mediated responses were now elicited by VLF stimulation in 1 WO preparations (middle) and by DR stimulation in a 2 WO animal (bottom middle). Administration of NT-3 (0.2 $\mu$g/ml) in 1 $\mu$M Mg$^{2+}$ solution enhanced all these NMDA responses (middle column). A decrease of extracellular Mg$^{2+}$ to 1 $\mu$M in the absence of the non NMDA antagonists (right column) enhanced the short latency AMPA/kainate receptor-mediated component of DR- (top right) and VLF-EPSPs in 1WO (middle right) and DR-EPSPs in 2 WO animals (bottom right). Addition of NT-3 enhanced these synaptic responses further. The amplitude of synaptic responses was measured at the vertical arrows.

GABA$_B$ receptor antagonists revealed two NMDA receptor-mediated synaptic responses (NMDA-m and NMDA-l). MK-801 passed directly into the motoneuron through the recording microelectrode blocked the NMDA-m response indicating that the NMDA receptors responsible for this EPSP are located in the motoneuron. The NMDA-m response is monosynaptic based on the finding that the stimulus intensity to elicit the maximum NMDA-m response and its ability to follow repetitive stimulation were similar to those for the monosynaptic AMPA/kainate-mediated response. The slightly higher latency for NMDA-m probably reflects the slower kinetics of NMDA receptors as compared with AMPA/kainate ones (Abdrachmanova et al. 2000).

In contrast, the NMDA-l component required a higher stimulus intensity and a lower rate of stimulation corresponding to values of these parameters necessary to elicit the long latency ventral root reflex recorded without pharmacological blockade in neonates (Thompson et al. 1993). This probably reflects the action of $\lambda$ fibers (Thompson et al. 1993) involving NMDA receptors on interneurons intercalated between dorsal roots and motoneurons although its abolition by MK-801 administered in the motoneuron indicates that the last order interneuron makes an NMDAergic synapse. These pharmacologically isolated NMDA receptor-mediated DR-evoked responses have been described previously (Pinco and Lev-Tov 1993; Ziskind-Conhaim 1990).

Here we show that NT-3, but not NGF, induces a marked facilitation of NMDA-mediated synaptic responses in motoneurons. We focus on the potentiation of NMDA-m because of the evidence that this is generated monosynaptically (see above) simplifying the interpretation of its mechanism of action. The simplest, most likely interpretation is that NT-3 directly potentiates the response of the postsynaptic NMDA receptor to glutamate since it has been shown to enhance the depolarizing response of motoneurons to bath-applied NMDA (Arvanov et al. 2000) and because the effect is eliminated by MK-801 in the motoneuron. However, it might also be argued that NT-3’s effect on the synaptically evoked NMDA response was to reduce presynaptic inhibition of transmission from spindle afferent fibers onto the motoneuron. This seems unlikely since MK-801 or BAPTA delivered intracellularly to the motoneuron in the absence of non NMDA receptor antagonists prevented NT-3 from enhancing AMPAR-mediated transmission from the same afferents (Arvanov and Mendell 2000). If NT-3 was reducing presynaptic inhibition of these afferents, AMPA/kainate receptor-mediated transmission should have been potentiated under these conditions. Thus we conclude that NT-3’s action on NMDA-m was localized to the motoneuron membrane. Although it is simplest to conclude that NT-3.

FIG. 5. Comparison of the effects of high Ca$^{2+}$ and low Mg$^{2+}$ in extracellular solution on the NMDA receptor-mediated synaptic response evoked by stimulation of DR and VLF in the same MN in P3 rat. (1) In control solution containing 2 mM Mg$^{2+}$/2.5 mM Ca$^{2+}$ in the presence of non-NMDAR antagonists stimulation of DR (400 $\mu$A, 50 $\mu$s, 0.01 Hz) evoked NMDA response. Under these conditions stimulation of VLF (500 $\mu$A, 500 $\mu$s, 0.01 Hz) failed to induce a synaptic response (this stimulus delivered to VLF evoked 4.7 mV peak amplitude response in this cell before administration of non-NMDA antagonists; not shown). (2) Increasing extracellular Ca$^{2+}$ to 4 mM (2 mM Mg$^{2+}$/4 mM Ca$^{2+}$) caused a larger DR-evoked NMDA response, but in high Ca$^{2+}$ solution stimulation of VLF was still unable to elicit a NMDA receptor-mediated response. (3) 15 min after perfusion of low Mg$^{2+}$ (1 $\mu$M Mg$^{2+}$/2.5 mM Ca$^{2+}$) solution stimulation of VLF began to evoke a synaptic response, similar to that evoked by DR stimulation in same cell. (4) APV (40 $\mu$M) completely blocks both DR- and VLF-evoked NMDAR-mediated responses in 1 $\mu$M Mg$^{2+}$ solution.
enhancement of later NMDAR-mediated components (NMDA-1) was also exerted via an action on postsynaptic NMDA receptors, the interneurons intercalated in this pathway makes it impossible to localize the site of NT-3 action definitively.

Interestingly, the effect of NT-3 on NMDA receptor-mediated transmission was transient since the amplitude of NMDA responses recovered to control values within about 20 min after washout of NT-3 (Fig. 3). In contrast, NT-3-induced enhancement of the monosynaptic AMPA/kainate receptor-mediated response, requiring motoneuron NMDA receptors to trigger it, persisted for at least 4 h after washout (Arvanov et al. 2000). We hypothesize that NT-3 enhances the response of postsynaptic NMDA receptors in motoneurons (Arvanov et al. 2000), which in turn triggers a long-lasting, possibly permanent increase in the response of AMPA/kainate receptors to synaptically released glutamate. Although these findings do not speak to the mechanism of NT-3’s interaction with NMDA receptors, neurotrophins have previously been suggested to affect NMDA receptors at central synapses either by interaction with the NR1 (Suen et al. 1997) and NR2B (Lin et al. 1998) subunits, or at the glycine site (Jarvis et al. 1997).

Consistent with previous findings from this laboratory (Arvanov et al. 2000), NT-3 was unable to facilitate monosynaptic AMPA/kainate receptor-mediated responses at DR-synapses from 2WO animals and at VLF-synapses on motoneurons. The latter are more mature, having previously been shown to form at least 1 wk earlier than DR synapses (Pincic and Lev-Tov 1994). Each of these NT-3-insensitive connections also lacked NMDAR-mediated synaptic responses (Figs. 1 and 3; Table 1). Postnatal decreases in NMDA receptor responses have been observed in various structures including the visual cortex (Car-mignoto and Vicini 1992; Tsumoto et al. 1987), the lateral geniculate nucleus (Ramoa and McCormick 1994), the thalamocortical synapse in somatosensory barrel cortex (Cair and Malenka 1995), and the superior colliculus (Hestrin 1992). At central glutamatergic synapses in rat the developmental decrease in the NMDAR component of the postsynaptic responses during postnatal days 12–18 is accompanied by a corresponding increase in the AMPAR component (Bellingham et al. 1998). The developmental reduction in the NMDAR-mediated current must involve a modification of the NMDA receptor complex itself since it is observed in excised membrane patches in response to glutamate application (Car-mignoto and Vicini 1992; Hestrin 1992).

The present study demonstrates that the developmental decrease in the NMDAR-mediated synaptic responses may result from the increased Mg2+ block of NMDAR in spinal motoneurons during the second postnatal week. These results are not inconsistent with previous reports of enhanced NMDA responsiveness in older neonates (Palecek et al. 1999) since those determinations were carried out in 0 Mg2+. The age-related reduction in NMDAR-mediated transmission demonstrated here is likely to reflect a developmental switch in subunit composition of the NMDA receptor (Flint et al. 1997; Monyer et al. 1994). Molecular cloning has identified several cDNA species encoding NMDA receptor subunits in neonatal rat motoneurons including NMDAR2A (NR2A), NR2B, and NR2C (Abdrachmanova et al. 2000). These display different sensitivity to Mg2+ with NR2A channels being more susceptible to block than NR2C channels (Monyer et al. 1992). We speculate that developmental switch between NR2B or NR2C and NR2A subunits of NMDAR, as occurs in other regions of the CNS (Adams et al. 1999; Pollard et al. 1993; Zhong et al. 1996), may determine the increased susceptibility to Mg2+ block of NMDAR in the postnatal rat spinal cord.

The timing of this developmental switch in the NMDA receptor’s functional properties suggests that it plays an important role in the development of the segmental reflex by determining the timing of NT-3’s effect on the AMPA/kainate current. The initial postnatal week is marked by a large increase in motoneuron size. Despite this the amplitude of the monosynaptic AMPA/kainate receptor-mediated EPSP remains roughly constant indicating a gain in synaptic strength (Seebach and Mendell 1996). The possibility that individual spindle afferent fibers make more profuse connections (i.e., more boutons and/or more release sites per bouton) on their target motoneurons as the spinal cord develops cannot be ruled out at present. Nonetheless, the long-lasting effect of brief NT-3 treatments on the monosynaptic EPSP via an LTP-like mechanism during this developmental stage (Arvanov et al. 2000) suggests that it might play a role in the maturation process. Indeed, we found that pulses of exogenous NT-3 delivered periodically during this time enhanced EPSP size, and trkC-IgG, which reduces endogenous levels of NT-3 (Ashkenazi et al. 1993), had a tendency to reduce it (Seebach et al. 1999). We do not know at present precisely where the NT-3 would come from in vivo but it is present in motoneurons (Buck et al. 2000; Johnson et al. 2000) and in glia (Dreyfus et al. 1999) and could presumably be released by these cells.

It is somewhat surprising that the effects of NT-3 on the amplitude of the monosynaptic EPSP were restricted to the motoneuron given the fact that trkC receptors are known to also be expressed on group Ia fibers (McMahon et al. 1994). NT-3 is known to have a presynaptic physiological effect at the neuromuscular junction (Yang et al. 2001) indicating a physiological action on motoneurons, although different from the one proposed in the present studies. It has further been suggested that neurotrophin trk receptors have access to several intracellular signaling systems through which they can elicit different effects including survival, growth and synaptic function (Kaplan and Cooper 2001). NT-3 has been demonstrated to encourage growth of group Ia fibers after damage (Mendell et al. 1999; Ramer et al. 2000) indicating that the role for NT-3 on afferent fibers may be quite different from that on motoneurons.

Recent experiments (Kerr et al. 1999) have demonstrated an increase in c-fos staining in dorsal horn neurons in response to intrathecal NT-3, very similar to that observed in response to intrathecal BDNF, both presumably due to activation of NMDA receptors in neurons of the dorsal horn. In these same experiments no effect of NT-3 was found on the late component of the ventral root reflex. These experiments were carried out in 12–14-day-old rat pups where the motoneuron NMDA receptors are largely inactive, and we speculate that this eliminated the ability of the motoneurons to respond to exogenous NT-3.

The present results suggest that re-establishing functional NMDA receptors might be required for neurons to regain sensitivity to NT-3. Presumably, lowering Mg2+ is not a practical way to accomplish this in vivo. However, if other approaches could be found, this might turn out to be a useful role for NT-3 in promoting recovery of the damaged spinal cord that complements its ability to promote regeneration of descending fibers (McTigue et al. 1998; Schnell et al. 1994).
REFERENCES


