Presynaptic Angiotensin II AT₁ Receptors Enhance Inhibitory and Excitatory Synaptic Neurotransmission to Motoneurons and Other Ventral Horn Neurons in Neonatal Rat Spinal Cord

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INTRODUCTION

The renin angiotensin system is now known to participate in a wide diversity of cells and tissues, sustaining homeostatic processes that include fluid and electrolyte balance and pressor effects. The latter are achieved via both circulating and central renin angiotensin systems. In the CNS, neurons and fibers display angiotensin-like immunoreactivity and have a widespread but discrete distribution, particularly prevalent in sites known to regulate neuroendocrine, cardiovascular, and autonomic systems (Lind et al. 1984). The octapeptide angiotensin (ANG II) and other angiotensin fragments are known to regulate neuroendocrine, cardiovascular, and autonomic systems (Lind et al. 1984). The octapeptide angiotensin (ANG II) and other angiotensin fragments are considered to function in neurotransmitter or neuromodulatory roles mediated by AT₁, AT₂, and AT₄ receptor subtypes (for reviews, see McKinley et al. 2003; Phillips and Summers 1998). A role for angiotensin receptors, in particular the AT₁ subtype, in regulating neuronal excitability can be inferred from electrophysiological and imaging observations of neuronal responsiveness to exogenously applied angiotensin peptides in a variety of neurons (e.g., Bai and Renaud 1998; Barnes et al. 2003; Ferguson and Washburn 1998; Gebke et al. 1998; Li et al. 2003; Oz and Renaud 2002; Shapiro et al. 1994; Yang et al. 1992). Binding studies and receptor-expression analyses also indicate a form of developmental plasticity within the central renin-angiotensin system with a high level of expression during the first and second postnatal week, changing during ontogeny (Milan et al. 1991; Tsutsumi and Saavedra 1991). Other studies suggest that ANG II receptors may have a trophic action, notably in spinal cord (Iwasaki et al. 1991) where fibers displaying ANG-II-like immunoreactivity and AT₁ receptors have been reported (Ahmad et al. 2003; Fuxe et al. 1976; Gehlert et al. 1986; Hosli and Hosli 1989; Lind et al. 1984; White et al. 1988).

Earlier extra- and intracellular observations revealing a depolarizing action of various peptides, including ANG II on neonatal rat lumbar motoneurons (Suzue et al. 1981) together with the availability of specific nonpeptide receptor antagonists prompted us to re-examine ANG II actions using patch-clamp techniques in neonatal rat spinal cord slice preparations. We recently reported that exogenous application of ANG II acts at postsynaptic AT₁ type receptors to engage two separate conductances that result in a prolonged membrane depolarization and inward current in a subpopulation of ventral horn motoneurons (Oz and Renaud 2002). The present analysis examined the origin of this latter feature. We now report that this property has two origins: a major component arises from an activity-dependent increase in synaptic activity due to the activation by ANG II of neurons that project to the recorded neurons; another less prominent component is activity-independent (tetrodotoxin-insensitive) and due to ANG II activation of receptors that are presynaptically located on the axon terminals of inhibitory and excitatory interneurons that synapse onto motoneurons.

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baseline thickening due to an increase in synaptic activity (top), loss of response 40 min later in the presence of Losartan (bottom). ANG II-induced increase in synaptic activity (top) and/or their morphology and evidence of an axon projecting toward the ventral root (Oz et al. 2001). After recording, slices were transferred to a fixation medium (4% paraformaldehyde with 0.1 mM phosphate buffer), stored overnight at 4°C, cleared for 60 min with dimethyl sulfoxide, fixed on microscope lamels, viewed, and measured under epifluorescence.

ANG II, a nonpeptide AT2 receptor antagonist PD123319, and tetrodotoxin (TTX) were obtained from Sigma-RBI (St. Louis, MO). Losartan, a nonpeptide AT1 receptor antagonist, was obtained from Merck (Rathway, NJ). Agents were dissolved in ACSF at their final concentrations and bath applied at a perfusion rate of 4–6 ml/min.

Data were analyzed with Clampfit software of pClamp versions 7 and 8 (Axon Instruments). For statistical evaluation, we used paired Student’s t-test or ANOVA as indicated (Origin version 6, Microcal Software, Northampton, MA). Results are presented as means ± SE. For assessment of spontaneous and miniature postsynaptic events, 3–4 min of recordings were sampled at 5 kHz and analyzed for frequency, amplitude, time to peak, and time constant of decay using a commercially available mini analysis software (Synaptosoft, Leonia, NJ). Spontaneous and miniature events were defined as those recorded in the absence and presence, respectively, of 1 μM TTX. Synaptic events were detected with an adjustable threshold set at 10–14 pA and maintained at a constant level in a given neuron. The analysis of mIPSCs and mEPSCs was performed with cumulative probability plots. Frequencies of synaptic events were calculated as the reciprocals of interevent intervals. Statistical comparisons of the frequency and amplitude of the synaptic currents before and after ANG II were made using the Kolmogorov-Smirnov (K-S) test; P < 0.05 was considered significant.

Ventral root recordings of spontaneous motoneuron activity

Experiments were performed on Swiss Webster mice (Taconic Laboratory), 1–4 days old (P0-3). The animals were anesthetized with methoxyflurane, decapitated, and eviscerated. The remaining tissue was placed in a dissecting chamber containing oxygenated (95% O2-5% CO2) low-calcium, high-magnesium, artificial cerebrospinal fluid [ACSF; concentrations (in mM) 128 NaCl, 4 KCl, 1.2 MgCl2, 2.4 CaCl2, and 10 d-glucose (pH 7.35; osmolality: 290–305 mosmol)] that was gassed with 95% O2-5% CO2. Membrane currents and potentials were continuously monitored on an oscilloscope and displayed on a recorder (Gould 2400S or Gould RS3200; Gould, Valley View, OH) and stored on videotape for off-line analysis. Digidata 1200 interface and version 7 of pClamp software were used on-line to generate current- and voltage-clamp commands.

Motoneurons were identified by their all-or-none antidromic responses to ventral rootlet stimulation applied with a concentric bipolar electrode (FHC, Bowdoinham, ME, tip diameter: 25 μm; 1–12 V; duration: 0.2 ms) and/or their morphology and evidence of an axon projecting toward the ventral root (Oz et al. 2001). After recording, slices were transferred to a fixation medium (4% paraformaldehyde with 0.1 mM phosphate buffer), stored overnight at 4°C, cleaned for 60 min with dimethyl sulfoxide, fixed on microscope lamels, viewed, and measured under epifluorescence.

FIG. 1. Angiotensin (ANG II) acting via AT1 receptors induces a prolonged enhancement in synaptic activity in motoneurons. A: whole cell current-clamp recording from a spinal motoneuron recorded on postnatal (PN) day 9 (resting membrane potential –68 mV). In response to a 30-s ANG II application (horizontal bar; estimated maximum concentration 1 μM), note the increase in baseline thickening due to an increase in synaptic activity (top). This response is markedly reduced in the presence of TTX (1 μM) in response to a 2nd application of ANG II in the same neuron 35 min later (bottom). Because EcL is approximately –64 mV with K-glucuronate-based internal pipette solutions, the contribution of the inhibitory events in this recording is negligible. B: Traces from voltage-clamp recordings (Vh –68 mV) in another cell illustrate ANG-II-induced increase in synaptic activity (top; inset illustrates data on faster time base), loss of response 40 min later in the presence of Losartan (middle) and partial recovery 45 min later after wash (bottom).
minimize the possibility of damage to the ventral roots. Then, the preparation was transferred to the recording chamber and superfused with oxygenated ACSF continuously re-circulated at room temperature.

Motoneuron electrical activity was recorded with plastic suction electrodes into which segmental L1-L3 ventral roots were drawn. Generally, recordings were made from two roots (left and right) of the same segment. The recordings were amplified (1,000 times), filtered (DC to 1 kHz), digitized at 2 kHz (Axon Instruments Digidata), and recorded on videotape for further analysis. ANG II (American Peptide, Vista, CA) was prepared as stock solutions of different concentrations and applied directly to the perfusate to give a final concentration of 30 or 300 nM.

The effects of ANG II on ventral root activity were quantified after band-pass filtering (100–2 kHz) and rectification. The rectified data were re-sampled at 100 Hz to minimize processing time. The total area under the rectified curve was computed for a 15-min period before the application of ANG II and a 15-min period immediately after drug application. The data were analyzed with Clampfit software of pClamp versions 7 and 8 (Axon Instruments), Origin 6.1 and using custom Matlab routines.

RESULTS

Motoneurons

Patch-clamp data obtained within Rexed laminae VIII and IX identified 102 motoneurons on the basis of their antidromic activation from ventral root stimulation and/or morphology. Cells displayed similar electrical properties with a mean resting membrane potential of −73.1 ± 1.2 mV and input resistance of 58.9 ± 5.6 MΩ. Consistent with earlier observations (see Oz and Renaud 2002), 38/102 cells responded to bath application of ANG II (0.3–1 μM; 30 s) with a slowly rising, prolonged and TTX-resistant postsynaptic depolarization or inward current. The present analysis focused on another feature, namely an ANG II-induced increase in baseline thickening observed under both current clamp and voltage clamp conditions in 50 neurons. Sensitivity to TTX (Fig. 1A; 4/4 cells) indicated that most of this synaptic activity was action potential dependent due to ANG II’s activation of neurons that were presynaptic to the recorded motoneuron.

To identify the nature of the receptors activated by ANG II, neurons displaying this feature were retested in the presence of AT1 and AT2 receptor antagonists. Because of the prolonged responses to ANG II and possible receptor desensitization, ≥30 min was allowed between successive ANG II applications. In 3/6 cells, the response to ANG II application was reversibly abolished when re-tested in the presence of the AT1 receptor antagonist Losartan (1 μM; see Fig. 1B) whereas the ANG-II-induced responses persisted in the remaining three cells when retested in the presence of PD123319 (1 μM). Thus these ANG-II-induced effects were mediated selectively by the peptide acting at AT1 type receptors.

Tests conducted in the presence of selective amino acid receptor antagonists clarified that these spontaneous events reflected input from both inhibitory and excitatory sources. In six cells tested in ACSF that contained 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (5 μM) and D-2-amino-5-phosphonovaleric acid D-APV (50 μM) to block ionotropic glutamate receptors, 1 μM ANG II induced a significant increase in both frequency (from 0.8 ± 0.3 to 8.2 ± 1.8 Hz) and amplitude (from 28.3 ± 3.4 to 54.7 ± 4.5 pA; P < 0.05, paired t-test, Fig. 2, A and B) of spontaneous inhibitory postsynaptic currents (sIPSCs). In three cells tested, these sIPSCs were completely abolished by addition of a cocktail of strychnine (2 μM) and bicuculline (20 μM; Fig. 2A). That most of these sIPSCs were glycineric rather than GABAAergic is implied by the observation of a dramatic reduction in response when tested only in the presence of strychnine at a concentration of 0.5 μM (n = 3 cells; not illustrated).

In five cells tested in ACSF containing strychnine (2 μM) and bicuculline (20 μM) to block glycineric and GABAA receptors, ANG II induced a significant increase in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs; from 1.1 ± 0.3 to 6.7 ± 2.1 Hz) without a significant increase in their amplitude (P < 0.01, paired t-test; Fig. 2, C and D). These observations imply that ANG II can activate both excitatory and inhibitory neurons that are presynaptic to motoneurons.

To address in more detail the presynaptic component of these responses, we examined the possible contribution of action potential-independent miniature events (mPSCs). In ACSF containing TTX (1 μM), NBQX (5 μM), and D-APV (50 μM) and pipettes containing 100 mM CsCl to amplify
mIPSCs, data from 7/13 cells revealed that ANG II (0.3 μM) significantly increased mIPSC frequency (0.6 ± 0.2 to 2.2 ± 0.5 Hz; paired t-test, P < 0.05), notably without a significant change in amplitude (Fig. 3, A–D) or kinetics (Fig. 3C, inset). In the remaining six cells, ANG II was without effect. Similar observations were made in 3/7 neurons assessed for an effect on mEPSCs where recordings used gluconate-based internal solutions and ACSF contained strychnine (2 μM) and bicuculline (20 μM). Response to 0.3 μM ANG II (n = 3) ANG II was an increase in mEPSC frequency (average of 0.9 ± 0.4 Hz in control vs. 2.8 ± 0.9 Hz after 0.3 μM ANG II) but not amplitude with no changes noted in the remaining four cells (not illustrated). These features from a subpopulation of neurons imply that ANG II may also influence action potential-independent transmitter release presumably by acting at receptors located on presynaptic terminals that derive from both inhibitory and excitatory interneurons.

Interneurons

In view of the substantial enhancement of synaptic events after ANG II, we next investigated the effects of ANG II on a population of 104 cells that were labeled as “interneurons” based on their lack of antidromic activation from ventral rootlet stimulation, their location and/or morphology. As a group, these cells differed significantly from motoneurons owing to their lower mean resting membrane potential of (−58.9 ± 5.6 mV; P < 0.05, ANOVA) and higher input resistance (183.1 ± 15.7 MΩ; P < 0.05, ANOVA).

As with motoneurons, responses to ANG II included both post- and presynaptic components. Because the former have been featured in an earlier report (Oz and Renaud 2002), the present analysis focused on a total of 57 cells that displayed an obvious ANG-II-induced increase in synaptic activity. In nine cells where ANG II applications in control ACSF induced a marked increase in spontaneous postsynaptic potentials, sensitivity to TTX (e.g., Fig. 4A) indicated that ANG II was exciting synaptic activity.

**FIG. 3.** ANG II increases the frequency of miniature synaptic currents. A: sequential sample current traces (V_{holding} = −67 mV) of miniature IPSCs (mIPSCs) from a motoneuron (PN 6 days) recorded with a pipette containing 100 mM CsCl in the presence of TTX (1 μM), NBQX (5 μM), and D-APV (50 μM). Top: controls; bottom: in the presence of ANG II (0.3 μM). B: cumulative distribution plots illustrate mIPSC interevent intervals during a control period (interrupted line) and the increase noted after application of ANG II (solid line). C: cumulative distribution plots of mIPSC amplitudes sampled indicate no significant difference between control and after ANG II. Inset: no change in kinetics of superimposed averaged traces of 20 consecutive mIPSCs recorded under control conditions and after ANG II. D: histograms of data from 6 responsive motoneurons illustrate mean mIPSC amplitude and frequency during control (C) and after ANG II (* P < 0.05).

**FIG. 4.** In interneurons, ANG II induces an enhancement of TTX-sensitive sIPSCs and sEPSCs. A: trace (V_{holding} = −61 mV) from an interneuron (PN 12 days) illustrates ANG-II-induced enhancement of synaptic events (details in the expanded trace). In this cell, a 2nd ANG II application 30 min later in the presence of 1 μM TTX was without an obvious response (bottom). Because E_{Cl} is approximately −64 mV with K-gluconate-based internal pipette solutions, the contribution of the inhibitory events in this recording is negligible. B: in ACSF containing strychnine (2 μM) and bicuculline (20 μM), current trace (V_{holding} = −63 mV) from another interneuron (PN 8 days) illustrates ANG-II-induced increase in sEPSCs. C: in ACSF containing NBQX (5 μM) and D-APV (50 μM), current trace from an interneuron (PN 11 days) recorded with 100 mM CsCl in the pipette illustrates an ANG-II-induced increase in sIPSCs.
cells that were presynaptic to the recorded neurons. Observations in the presence of amino acid receptor antagonists confirmed contributions from both inhibitory and excitatory synaptic events (Fig. 4, B and C). Spontaneous IPSCs responded to ANG II (1 μM) with a significant increase both in frequency (from 1.1 ± 0.4 to 9.8 ± 2.6 Hz; P < 0.01) and amplitude (from 26.2 ± 3.9 to 44.8 ± 5.7 pA; P < 0.05; n = 5 cells). Similarly, spontaneous EPSCs exhibited a significant increase both in frequency (from 1.4 ± 0.3 to 12.9 ± 3.4 Hz; P < 0.01) and amplitude (from 18.9 ± 2.8 to 34.6 ± 4.7 pA; P < 0.05; n = 4 cells).

Further tests in the presence of TTX identified a small but detectable contribution of miniature postsynaptic events. In ACSF containing glutamate receptor antagonists, recordings in 9/19 cells tested using pipettes containing 100 mM CsCl revealed that ANG II induced a significant increase in mIPSC frequency (from 0.6 ± 0.2 to 2.4 ± 0.7 Hz; P < 0.05, paired t-test) without a change in amplitude (Fig. 5, A–D). In the remaining 10 cells, ANG II was without effect. In ACSF containing strychnine (2 μM) and bicuculline (20 μM), recordings in 8/17 cells recorded with gluconate-based internal solutions revealed a significant ANG-II-induced increase in mEPSC frequency (from 1.2 ± 0.4 to 3.8 ± 0.7 Hz; P < 0.05, paired t-test) without change in amplitude (Fig. 6, A–D).

FIG. 5. ANG II enhances the frequency of mIPSCs in interneurons. A: in ACSF containing TTX (1 μM), NBQX (5 μM) and t-APV (50 μM), consecutive current traces (Vh = −62 mV) in an interneuron (PN 12 days) recorded with 100 mM CsCl in the pipette illustrate controls (top) and ANG-II-induced increases in mIPSC frequency (bottom). B and C: corresponding cumulative distribution plots for interevent intervals and amplitude illustrate a significant rise in mIPSC frequency but no change in amplitude after exposure to ANG II (solid lines). D: Histograms of these data from 7 cells illustrate enhancement in frequency without a change in amplitude of mIPSCs (* P < 0.05).

Thus analogous with data from motoneurons, these events indicate two components to the “presynaptic” actions of ANG II. The most prominent of these is TTX sensitive, reflecting an action at somatic and/or dendritic receptors on neurons that synapse onto interneurons. Interestingly, under resting conditions, it would appear that many of the synapses activated by this mechanism are silent. The other action is TTX resistant, reflecting an effect on transmitter release from axon terminals of inhibitory and excitatory neurons that synapse onto interneurons.

Effects of ANG II on spontaneous motor activity recorded from the ventral roots

To assess whether these events related to functionally relevant spinal cord outputs, the effects of ANG II on motor output were examined in the isolated spinal cord of the neonatal mouse. Under control conditions, we recorded spontaneous depolarizations and discharge from the lumbar ventral roots (n = 7) as described in an earlier report (Whelan et al. 2000). Figure 7 shows that bath-application of ANG II (30–300 nM) increased the amplitude and discharge of spontaneous depolarizations recorded from the lumbar sacral ventral roots. The effects were dose dependent and the largest changes were observed at 300 nM. The changes were quantified by measuring the area under the slow ventral root potentials over a 15-min period before and during bath application of ANGII.
The area increased by ~20% (30 nM) and by ~75% (300 nM) of the normalized control level.

DISCUSSION

In an earlier study, we reported that ANG-II-induced activation of postsynaptic AT$_1$ receptors could induce membrane depolarization and inward current in subpopulations of neonatal spinal thoracolumbar motoneurons and ventral horn interneurons (Oz and Renaud 2002). The present analysis focused on another feature that was commonly associated with these ANG-II-induced responses, namely an increase in baseline synaptic activity. In the presence of specific amino acid receptor antagonists, we observed that this activity involved both inhibitory (GABAergic and glycineric) and excitatory (glutamatergic) synaptic activity. The large reduction of this “presynaptic” activity in media containing TTX indicates that it is probably due to ANG-II-induced membrane depolarization and action potential generation in inhibitory and excitatory neurons that project to the recorded neurons. However, the detection of a TTX-resistant ANG-II-induced increase in mEPSCs and mEPSCs without a change in their amplitude leads to the conclusion that angiotensin receptors are also located on presynaptic terminals where they can influence action potential-independent transmitter release at inhibitory and excitatory synapses on ventral horn neurons.

The release of a retrograde messenger such as NO is a possible mediator of enhanced synaptic events (Ludwig and Pittman 2003). Synthesis of such a retrograde messenger can be sequentially linked to postsynaptic depolarizations, opening of voltage-gated Ca$^{2+}$ channels, and a subsequent increase of intracellular Ca$^{2+}$ levels. In those neurons where no postsynaptic depolarization is detected yet there is a change in synaptic events, it is unlikely that a retrograde messenger is produced. However, it is conceivable that under these circumstances an ANG-induced change in the occurrence of synaptic events may still arise because of diffusion of a retrograde messenger from neighboring cells that are depolarized.

Although these spinal cord angiotensin receptors may undergo some developmentally regulated changes (Milan et al. 1980), it is apparent from the present observations that they are functional in the early postnatal period. Evidently these receptors persist into adult life based on previous studies revealing widespread [125I]ANG II binding (Holsi and Holsi 1989; White et al. 1988) and extracellular electrophysiological recordings of saralasin-sensitive angiotensin-induced excitation in adult spinal cord neurons (White et al. 1988). The present observations also provide a functional counterpart for the recently reported ultrastructural immunohistochemical observations that angiotensin receptors can be localized to both somatic and dendritic membranes as well as synaptic boutons in adult rat spinal cord (Ahmad et al. 2003).

At postsynaptic AT$_1$ receptors where it is possible to analyze mechanisms of action, angiotensin can be seen to induce changes in cell excitability via modulation of potassium and/or calcium conductances by various signaling mechanisms (reviewed in Sumners et al. 2002). By contrast, although some reports have shown that presynaptic angiotensin receptors can attenuate rapid neurotransmitter release (e.g., Barnes et al. 2003; Li et al. 2003) restricted access has hindered analysis of mechanisms whereby presynaptic AT$_1$ receptors influence transmitter release.

Neonatal spinal motoneurons are depolarized by activation of a variety of peptide receptors (cf. Suzue et al. 1981), and the present results add to an expanding literature that confirms the presence of multiple functional peptide receptors in the developing spinal cord, e.g., thyrotropin-releasing hormone (Kolaj et al. 1997), vasopressin and/or oxytocin (Kolaj and Renaud 1998; Oz et al. 2001; Pearson et al. 2003), and substance P (Ptak et al. 2000). An issue that remains to be addressed is the endogenous source for any of these peptides. A hypothalamic origin is possible because fibers from the paraventricular nucleus apparently reach the thoracic cord at prenatal age and the lumbar cord by postnatal day 2 (Kudo et al. 1993; Lakke 1997; Leong et al. 1984). Although, it is not known whether these fibers contain ANG in neonatal spinal cord, angiotensin-like immunoreactivity has been noted in paraventricular nuclei neurons that project to autonomic regions in the adult rat spinal cord (Galabov et al. 1990; Lind et al. 1984). Another possibility may derive from the widespread synthesis and constitutive secretion of angiotensinogen, the only precursor of ANG II, by neuronal and glial cells (see review by Semia 1995). Brain angiotensinogen levels and expression are high during late fetal and neonatal development (Gomez et al. 1988; Sood et al. 1990a,b) and angiotensin converting enzyme reaches maximum expression levels at 2 wk after birth (Strittmatter et al. 1986). There is a transient up-regulation of the renin-angiotensin system in the neonatal period (Kalinyak et al. 1991; Wallace et al. 1980) and plasma concentration of ANG II increases transiently about fivefold in the neonatal (Wallace et al. 1980). Collectively these observations support a role for angiotensin receptors in neuronal growth and development, a feature noted in various preparations, including cultured hypothalamic neurons (Jirikowski et al. 1984); peri-implantation rat
embryo (Tebbs et al. 1999), and embryonic neurons (Ikeda et al. 1989; Iwasaki et al. 1991; Sood et al. 1990b). In addition, angiotensin belongs to a family of vasoactive and mitogenic peptides that participate in physiological and pathological cell growth and differentiation (Van Biesen et al. 1996) and can activate a set of kinases that are known for their importance in cell survival (Richards et al. 1999; Yang et al. 2001).

Ang II administration increased the frequency of spontaneous depolarizations and discharge recorded from the ventral roots. Because the ventral roots contain preganglionic autonomic and somatic motoneuronal axons, ventral root recordings will reflect activity from both sources. However, we believe that somatic motoneuronal activity provides the dominant contribution to the slow ventral root potentials. This is because the slow potentials are electrotonically propagated population synaptic potentials generated in neuronal somata and proximal dendrites. The preganglionic autonomic somata are ~100–200 μm further than motoneuronal cell bodies from the recording site at the ventral roots; this will result in a correspondingly greater attenuation of their slow potentials. This conclusion is in agreement with the results of Pearson et al. (2003) who found that two other peptides, vasopressin and oxytocin, can increase the frequency and amplitude of somatic motoneuronal discharge in the neonatal mouse cord. Our results, therefore demonstrate that ANG II can modulate the functional output of spinal networks during the neonatal period when locomotor networks are becoming functional. It remains to be determined if their actions during this period can influence network assembly, either indirectly by altering activity or directly through their trophic effects.

Several lines of evidence indicate that synaptic activity is an important component of motoneuron development. Motoneuron differentiation is delayed in rat embryonic spinal cords cultured in the presence of TTX, indicating that electrical activity influences the time course of their development (Xie and Ziskind-Conhaim 1995). Similar to TTX action, motoneuron development is retarded when synaptic release is chronically blocked with tetanus toxin or omega-conotoxin, a Ca2+ channel blocker. Moreover, incubating spinal cords in medium additionally blocked with TTX, indicating that electrical activity modulates motoneuron differentiation via Ca2+-dependent synaptic release of neurotransmitters or neurotrophic factors (Xie and Ziskind-Conhaim 1995). Indeed, activity-dependent developmental processes have been demonstrated to have profound effects not only on synaptic connectivity but also on the morphological and electrophysiological properties of motoneurons (for a review, Kalb and Hockfield 1992).

Further experiments on the endogenous source of ANG II and functional role of ANG II receptors in development of neonatal spinal cord may provide some insight on these issues.

REFERENCES


