Rostrocaudal Progression in the Development of Periodic Spontaneous Activity in Fetal Rat Spinal Motor Circuits In Vitro

KIYOMI NAKAYAMA,1,2 HIROSHI NISHIMARU,1 MAKITO IIZUKA,2 SHIGERU OZAKI,1 AND NORIO KUDO1
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Nakayama, Kiyomi, Hiroshi Nishimaru, Makito Iizuka, Shigeru Ozaki, and Norio Kudo. Rostrocaudal progression in the development of periodic spontaneous activity in fetal rat spinal motor circuits in vitro. J. Neurophysiol. 81: 2592–2595, 1999. Developmental changes in the periodic spontaneous bursts in cervical and lumbar ventral roots (VRs) were investigated using isolated spinal cord preparations obtained from rat fetuses at embryonic days (E) 13.5–18.5. Spontaneous bursts were observed in the cervical VR at E13.5–17.5, and in the lumbar VR at E14.5–17.5. Bursts occurrence in the cervical and lumbar VRs was correlated in a 1:1 fashion at E14.5–16.5. The bursts in the cervical VR preceded those in the lumbar VR at E14.5, but the latter came to precede the former by E16.5. The interval between spontaneous bursts in the lumbar VR was greatly prolonged after spinal cord transection at the midthoracic level at E14.5, whereas that in the cervical VR became significantly longer at E14.5–16.5. These results suggest that the dominant neuronal circuit initiating the spontaneous bursts shifts from cervical to lumbar region during this period. Bath application of a glutamate receptor antagonist, kynurenic acid (4 mM), had little effect on the spontaneous bursts in either cervical or lumbar VRs at E14.5–15.5. At E16.5, kynurenic acid abolished the spontaneous bursts in the cervical VR. Concomitant application of kynurenic acid and strychnine (5 μM), a glycine receptor antagonist, abolished all spontaneous bursts, suggesting that the major transmitter mediating the spontaneous bursts changes from glycine to glutamate in the cervical region by E16.5, but not in the lumbar region during this period.

INTRODUCTION

In rats, periodic spontaneous motoneuronal activity is observed in the lumbar spinal cord during the last week before birth, a time that coincides with the period during which formation of the spinal neuronal networks accelerates (Kudo and Yamada 1987; Ozaki et al. 1996). This spontaneous activity is likely to be generated at this time as a result of the temporary excitatory effects of glycine and γ-aminobutyric acid (GABA) (Nishimaru et al. 1996), suggesting that the spontaneous activity might be playing an important role in the activity-dependent development of the neuronal network. Moreover, this activity may be responsible for the spontaneous limb movements observed at the same period in utero (Narayanan et al. 1971). However, the developmental profile and the nature of the correlation between forelimb and hindlimb activity both remain unclear. Our present results demonstrate that as early as embryonic day 14.5 (E14.5) independent neuronal mechanisms in the cervical and lumbar regions serve to evoke periodic spontaneous motoneuronal activity and that these mechanisms are highly interactive. Parts of this study have been published in abstract form (Ozaki et al. 1990).

METHODS

Isolated spinal cord preparations from rat fetuses aged E13.5–18.5 were obtained as previously described (Nishimaru et al. 1996). The ventral roots (VRs) from the C6–C8 and L2–L5 segments were cut and incorporated into glass suction electrodes. The preparation was placed in an experimental chamber perfused with normal Krebs solution (composition in mM: 118.4 NaCl, 4.69 KCl, 2.52 CaCl2, 1.25 MgSO4, 25.0 NaHCO3, 1.18 KH2PO4, and 11.1 glucose) saturated with 95% O2–5% CO2 (pH 7.3–7.4 at room temperature). In 17 preparations, the isolated spinal cord was further transected at the thoracic level during the course of the experiment. The drugs kynurenate (Sigma) and strychnine (Wako Chemicals) were applied by addition to the perfusate. Electrical activity in the VRs was amplified using AC-coupled amplifiers (gain: 10 k, band-pass filter: 15 Hz to 3 kHz; Nihon Kohden). Parts of the records were integrated with a time constant of 0.5 s. The burst duration and the interval between spontaneous bursts were measured over 10–20 cycles and are given as means ± SD. The significance of differences was determined using a Student’s t-test.

RESULTS

Motoneuronal activity was recorded in cervical and lumbar VRs using isolated spinal cords from E13.5–18.5 rat fetuses (n = 67). Periodic spontaneous bursts could be recorded in the cervical VR as early as E13.5 (n = 4; Fig. 1Aa), but they were first recorded in the lumbar VR at E14.5. Such activity showed a 1:1 correlation between cervical and lumbar VRs in all preparations examined at E14.5 (n = 16) and at E15.5 (n = 16), and in 19 of 22 preparations at E16.5 (Fig. 1, Ab and Ac), indicating a degree of temporal synchronization between the two regions. In fact, the activity in the cervical VR tended to precede that in the lumbar VR at E14.5 (Fig. 1, Ba and Bd). However, by E16.5, most bursts of VR activity in the lumbar region preceded the corresponding burst in the cervical region (Fig. 1, Bc and Bd). The temporal relationship between the bursts in cervical and lumbar VRs was less consistent at E15.5 (Fig. 1, Bb and Bd). The mean delay between the activity in the cervical VR and that in the lumbar VR was 1.6 ± 1.1 (SD) s at E14.5 (n = 6), 0.5 ± 1.6 s at E15.5 (n = 6) and −2.4 ± 1.8 s at E16.5 (n = 6). Some of the bursts in the lumbar VR failed to entrain bursts in the cervical VR in 3 of 22 preparations at E16.5 and in 3 of 5 preparations at E17.5 (Fig. 1Ad). In two preparations at E17.5 and in all preparations at E18.5 (n = 4;
Fig. 1Ae), no periodic spontaneous bursts were observed in either VR. These results suggest 1) that neuronal connections exist to coactivate the motoneurons periodically in these two limb-innervating regions during this period of development and 2) the spontaneous burst starts from the rostral region at early stages and from the caudal region at later stages.

To examine the relative ability of the cervical and lumbar spinal cord to initiate spontaneous bursts, the spinal cord was transected at the midthoracic level (T7–T8) at E14.5–16.5. Spontaneous bursts could still be observed in both cervical and lumbar VRs after this transection in all preparations (Fig. 2). The effects of the transection on burst interval and burst duration are summarized in Table 1. The interval between spontaneous bursts was significantly (P < 0.05) prolonged in the cervical and lumbar VRs by such transection at E14.5 (Fig. 2A; Table 1). Moreover, the interval between spontaneous bursts became longer in the lumbar VR than in the cervical VR (P < 0.05). At E15.5, the transection had differential effects: it prolonged the burst interval in the cervical VR, but did only slightly and nonsignificantly affect the lumbar VR burst interval (Fig. 2B; Table 1). After transection at E16.5, the interval between the spontaneous bursts recorded from the cervical VR was highly variable, and the mean interval value was significantly increased (P < 0.05, Fig. 2C; Table 1). At this stage, the spontaneous bursts recorded from the lumbar VR were not affected at all.
The duration of the individual bursts was little affected by transection at E14.5–15.5 (Table 1). At E16.5, before transection the correlated spontaneous bursts had a variable time course with some having more than two peaks and a long duration of >40 s (Fig. 2C). However, such bursts were found only in the cervical VR after the transection, whereas the burst duration became less variable in the lumbar VR. The results of the transection experiment indicate that both the cervical and lumbar regions of the spinal cord are capable of generating spontaneous activity during this period of development.

We examined the effect of the broad-spectrum glutamate receptor antagonist, kynurenate (4 mM), on the correlated spontaneous bursts in the cervical and lumbar VRs at E14.5–16.5. Bath application of this antagonist failed to abolish the spontaneous bursts recorded from the cervical and lumbar VRs at E14.5 (n = 7; Fig. 3A) and E15.5 (n = 5). In contrast, at E16.5 the spontaneous bursts in the cervical VR were abolished by kynurenate in 11 of 12 preparations. The spontaneous bursts reappeared after 7–25 min (while kynurenate was still present) in 7 of 11 preparations (Fig. 3B). In the lumbar VR, however, the spontaneous bursts persisted in the presence of kynurenate in all 12 preparations examined. Spontaneous bursts with variable time course and long duration were not observed in the lumbar VR when glutamate receptors were blocked. Bath application of kynurenate together with the glycine receptor antagonist, strychnine (5 μM), blocked the spontaneous bursts in the cervical and lumbar VRs at all embryonic days examined (n = 5 at E14.5; n = 4 at E15.5; n = 8 at E16.5; Fig. 3, A and B). These results indicate that glutamate becomes the dominant transmitter responsible for generating spontaneous bursts in the cervical spinal cord by E16.5, while glutamate becomes dominant in the lumbar spinal cord at E17.5 (Nishimaru et al. 1996). It is thus likely that neuronal circuits generating periodic spontaneous bursts develop earlier in the rostral than in the caudal parts of the spinal cord.

### TABLE 1. Interval and burst duration for spontaneous bursts recorded before and after spinal cord transection at T7–T8

<table>
<thead>
<tr>
<th>Age</th>
<th>Cervical VR</th>
<th>Lumbar VR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Transected</td>
</tr>
<tr>
<td></td>
<td>E14.5</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>E15.5</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>E16.5</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>E14.5</td>
<td>6.6 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>E15.5</td>
<td>6.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>E16.5</td>
<td>9.2 ± 6.3</td>
</tr>
</tbody>
</table>

Results are means ± SD; n is the number of preparations examined. VR, ventral root; E14.5, embryonic day 14.5. *P < 0.05 compared with intact; Student’s t-test.

DISCUSSION

The present study has revealed correlated spontaneous burst activity in the cervical and lumbar VRs in rat fetuses at and after E14.5. Periodic spontaneous body movements having a close correlation between forelimb and hindlimb at and after E12.5 (Suzue 1996). The duration of each episode and the interval between episodes, as well as the delay between the onset of forelimb and hindlimb movements, are in a similar range to the corresponding values obtained for the spontaneous burst activity recorded in the present study. This is consistent with the idea that the neuronal activity described in this study may generate the periodic spontaneous body movement shown by the fetus in utero.

The present study shows that the location of the neuronal circuit initiating the periodic spontaneous bursts shifts during development from the cervical to the lumbar region in the fetal rat spinal cord, as is the case for the neurogenesis of motoneurons (Nornes and Das 1974) and for the formation of cutaneous reflexes (Narayan et al. 1971). The results of this study suggest that at E14.5 the major excitatory drive for such neuronal circuits is likely to be provided by glycine-mediated synapses throughout the whole spinal cord, although spontaneous bursts are typically initiated in the cervical region. At E16.5, however, the dominant excitatory transmitter has become glutamate in the cervical region, although it is still glycine in the lumbar region. The ability to initiate the spontaneous motor activity was greater in the lumbar spinal cord at this stage. Thus it is suggested that the immature neuronal circuits, which contain mainly glycine-mediated excitatory synapses, are more excitable than those in the region in which glutamate has become the dominant excitatory transmitter. These immature neuronal circuits may promote the rostrocaudal development of spinal neuronal networks, perhaps by supporting neuronal differentiation.

A recent study in the neonatal rat spinal cord has shown that strychnine can antagonize the action of GABA (Jonas et al. 1998). However, GABA<sub>A</sub> receptors are likely to be less involved in the excitatory pathways underlying the present type of spontaneous activity because concomitant application of
kynurenate and bicuculline (20–40 μM), a GABA<sub>A</sub> receptor antagonist, does not abolish the spontaneous bursts in the cervical (n = 4 at E14.5; K. Nakayama, unpublished observation) or lumbar cord (Nishimaru et al. 1996).

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