Alpha-1 Adrenoceptor Agonists Generate a “Fast” NMDA Receptor-Independent Motor Rhythm in the Neonatal Rat Spinal Cord

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INTRODUCTION

Monoamines have long been known as potent modulators of rhythmic motor networks. Lundberg and colleagues have shown that L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine and noradrenaline, initiates coordinated rhythmic activity in spinal cats (Jankowska et al. 1967; for review, see Hultborn et al. 1998). This rhythmic activity exhibited complex motor patterns controlled by descending projections from the locus coeruleus and the subcoeruleus nucleus, and its terminations are distributed throughout most of the spinal gray matter with the highest densities at the superficial dorsal horn, the thoracolumbar intermediolateral column, and the ventrolateral ventral horn (Clark and Proudfoot 1991a,b; Fuxe et al. 1990; Grzanna and Fritschy 1991; Martin et al. 1999; Westlund et al. 1982).

Although it is clear that monoamines are important regulators of locomotion in the adult spinal cord, their role in the neonatal spinal cord is less clear. Although many noradrenergic terminals are present in the cervical and lumbar cords of rats during the first postnatal week (Rajaofetra et al. 1992; Tanaka et al. 1996), it has been difficult to establish a clear functional role for NA or adrenoceptor agonists in locomotion. For example, bath application of NA failed to produce a locomotor rhythm in the neonatal rat spinal cord preparation (Kiehn et al. 1999; Sqalli-Houssaini and Cazalets 2000). Interestingly, dopamine, the precursor of NA has been shown to induce a slow and organized motor pattern in the neonatal rat spinal cord (Kiehn et al. 1996; Smith et al. 1988), and its presence facilitates the expression of the locomotor rhythm in isolated spinal cords of young adult mice (Jiang et al. 1999a; for review see Bonnot et al. 2002b).

The apparent differences in the response of the adult and neonatal cord to adrenoceptor agonists may reflect the relative immaturity of the central pattern generators in the younger cords. However, we now show, in contrast to earlier studies, that adrenoceptor agonists can produce a transient rhythm with a 1- to 4-s cycle time and an organized alternating-left-right pattern in rostral lumbar and sacrococcygeal segments of the neonatal rat spinal cord. In this paper, we investigate the receptors associated with the generation of the rhythm, its segmental origin, the organization of the networks involved in its generation, and the induced motor behavior. Our findings provide further insights into the maturation of vertebrate locomotor pattern generating circuitry.

METHODS

Preparations

Spinal cord preparations (T6–Co3) were isolated from P2–P4 ether-anesthetized rats with or without an intact tail (Delvolve et al. 2001; the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact. Address for reprint requests and other correspondence: A. Lev-Tov, Dept. of Anatomy and Cell Biology, The Hebrew University Medical School, PO. Box 12272, Jerusalem 91120, Israel (E-mail: aharony@md.huji.ac.il).
Lev-Tov and Delvové 2000; Lev-Tov et al. 2000). The cord was transferred to a recording chamber and superfused continuously with an oxygenated Krebs saline (e.g., Delvové et al. 2001; Kremer and Lev-Tov 1997; Lev-Tov et al. 2000).

Stimulation and recordings

Ventral root potentials (VRPs) were recorded by suction electrodes from pairs of lumbar and sacral ventral roots (DC or 0.1 Hz to 10 kHz) using a high gain DC/AC amplifier. Sharp electrode intracellular recordings were obtained from L2, L5, and S1 motoneurons impaled from the ventral or ventrolateral aspect of the cord and identified by the presence of antidromic spikes. Microwire EMG recordings (100 Hz to 10 kHz) were obtained from left and right tail flexors and extensors (see Delvové et al. 2001; Gabbay et al. 2002; Lev-Tov et al. 2000). Rhythmic activity was induced by bath application of NA or the α1 adrenoceptor agonist methoxamine or phenylephrine.

Data acquisition and statistical analysis

Recorded data, were digitized (Digidata 1320A, Axon Instruments), and stored on the computer’s hard disk for subsequent analyses (see Gabbay et al. 2002; Strauss and Lev-Tov 2003). VRP data were low-pass filtered at 50–100 Hz and analyzed using either Clampfit 9 (Axon Instruments) or the time series routines of STATISTICA 6 (StatSoft 2001). A detailed description of the time series analysis can be found in Strauss and Lev-Tov (2003). Briefly, rhythmic data were divided into equal consecutive segments, the frequency of the rhythm, and the phase shift between any given pair of time series variables were extracted using Fourier bivariate (cross spectral) analysis. The cycle time data were analyzed using linear statistical methods. Data were pooled when required if one-way ANOVA revealed no significant differences between the data samples. The phase data were analyzed using circular statistics to calculate the mean phase-lag and the r vector describing the concentration of phase-lag values around the mean under each experimental condition (Delvové et al. 2001; Gabbay et al. 2002; Lev-Tov et al. 2000). Data were pooled when required if the Watson and Williams test revealed no significant differences between the tested samples. The Rayleigh’s test (Zar 1984) was used to determine whether the phase values were uniformly distributed around the circle (see Delvové et al. 2001; Gabbay et al. 2002). Multi-sample testing was performed to compare the mean phase values of any pair of tested factors (the Watson-Williams test) (Zar 1984).

RESULTS

NA induces a “fast” alternating rhythm in lumbar and sacrococcygeal segments of the spinal cord

NA has been reported to produce irregular bursting or a very slow rhythm in the lumbar cord of neonatal rats (Kiehn et al. 1999; Sqalli-Houssaini and Cazalets 2000). Figure 1 shows that NA also produced a “fast” alternating-left-right rhythm in lumbar and sacral segments of the spinal cord (A). Analysis of this rhythm revealed a mean cycle time of 2.8 ± 1.2 (SD) s, a robust alternating pattern with a mean left-right phase shift (ΦL-R) of 0.51 ± 0.09 cycles, r vector = 0.84 (7 experiments), and a coupled activity of the rostral-lumbar and ipsilateral sacrococcygeal segments with a mean phase shift (ΦL2-LS2) of 0.92 ± 0.07 cycles (r vector = 0.92, 6 experiments).

Intracellular recordings from an S2 motoneuron (Fig. 1B) show that the “fast” rhythm produced by NA is expressed as rhythmic oscillations of transmembrane potential superimposed on a tonic depolarization (~20 mV) and that the bursts of spikes elicited during the depolarizing phases of these oscillations were in phase with the ipsilateral ventral root firing. The fast rhythm induced by NA was short-lasting, it slowed down within 1–2 min, decomposed into irregular packets of short alternating bursts mixed with occasional longer bursts (Fig. 1C), and finally (~5 min) blocked.

Fast rhythm is mediated mainly by activation of α1-adrenoceptors

The pharmacological basis of the fast NA-induced rhythm was examined in a series of eight experiments. The fast rhythm (Fig. 2A) could be induced when NA was added to the bath 20 min after application of the α2-adrenoceptor blocker yohimbine (1 μM, Fig. 2B). The rhythm persisted in the presence of 2, 5, and 10 μM yohimbine and, in some of the experiments, in the presence of 20 μM of the drug and exhibited various perturbations when the concentration of yohimbine exceeded 20 μM. By contrast, application of the α1-adrenoceptor blocker prazosin (1 μM) to the preparation prevented the ability of NA to initiate the rhythm (Fig. 2C). Similar results were obtained in each of the experiments performed in this series. These results suggested that the fast NA-induced rhythm is accounted for mainly by activation of α1-adrenoceptors, a suggestion that was further supported by the finding that the fast rhythm could be initiated by bath application of the α1-adrenoceptor agonists methoxamine (Fig. 2D) or phenylephrine (not shown). The methoxamine-induced rhythm was superimposed on a tonic depolarization (11.7 mV, this experiment; mean = 13.8 ± 5.4 mV, n = 18, pooled methoxamine and NA data) and was accompanied by a significant increase in input resistance (48.2 ± 21.3%, sampled during nonrhythmic epochs in the 5 tested cases, P < 0.01, 2-tailed t-test). The cycle time of the methoxamine induced rhythm was 2.7 ± 0.85 s, and the left-right phase was 0.5 ± 0.001 cycles, r vector = 0.89 (10 experiments). Unlike the NA-induced rhythm, the rhythm produced by methoxamine was long-lasting. It slowed down gradually and persisted for tens of minutes (30–60 min). To test whether the rhythm produced by α1-adrenoceptors required an activation of NMDA receptors, we tried to induce the rhythm by methoxamine in the presence of the NMDA receptor blocker 2-amino-5-phosphonopentanoic acid (AP5, 200 μM).

Figure 3 shows that a robust rhythmic activity could be produced in S2 (B) as well as L2 (C) by methoxamine in the presence of AP5. The cycle time of the rhythm was slower than the control rhythm (5 experiments; histograms, D, P < 0.001, 2-tailed t-test) and its alternating-left-right pattern was unchanged (Watson and Williams test for multiple comparisons), circular diagrams (D, control: ΦL2-RL2 = 0.49 ± 0.02 cycles, r vector = 0.83, dLS2-RS2 = 0.49 ± 0.09 cycles, r vector = 0.86; AP5: ΦL2-RL2 = 0.51 ± 0.1 cycles, r vector = 0.81, dLS2-RS2 = 0.47 ± 0.1 cycles, r vector = 0.83, respectively, 5 experiments).

Pattern and segmental source of the methoxamine-induced rhythm

In a previous study, we have shown that NA induced rhythmic tail movements characterized by left-right alternations of the tail musculature and by coactivation of flexors, extensors, and abductors on a given side of the tail (Gabbay et al. 2002). Similar movements and patterns were induced by
bath-applied methoxamine (not shown). To determine the pattern of flexor-extensor activation at the lumbar cord during the rhythm, we recorded the activity produced by methoxamine from flexor (L₁–L₄-) and extensor (L₅-) dominated segments of the lumbar spinal cord. Recordings from the left L₂–L₅ ventral roots and from the left and right S₂ ventral roots are shown in Fig. 4. Immediately (2–3 min) after addition of methoxamine to the bath, fast rhythmic bursts appeared in L₂–L₄ and S₂ (cycle time: 3.14 ± 0.4 s, 5 experiments) but not in L₅ (A). Analyses of the temporal relation among L₂, L₃, and S₂ (5 experiments) revealed that φL₂-L₅ was 0.96 ± 0.003 cycles, r vector = 0.99, meaning that the L₂ rhythm lagged by 125 ± 8 ms after that of the ipsilateral S₂, thereby, suggesting a caudorostral spread of the rhythm. The phase shift between L₂ and L₃ (φL₂-L₃) was 0.003 ± 0.0001 cycles (r vector = 0.99), and the phase shift between the left and right S₂ (φL₅-L₅) was 0.49 ± 0.001 cycles, r vector = 0.98. At a later stage, (5–10 min after addition of methoxamine, B), the fast rhythm in the flexor-dominating lumbar segments was accompanied by a superimposed slow rhythm (B, ↓). The slow rhythm that had a cycle time of 64 ± 34.4 s (data pooled from 5 experiments in this series and 9 additional experiments)
compares to 3.14 ± 0.4 s, 5 experiments of the fast rhythm (see histograms in C), appeared in all the recorded lumbar (L2–L5) and sacral segments, exhibiting ipsilateral synchronicity of flexor- and extensor-dominated segments of the lumbar cord (dL2–L5 = 0.02 ± 0.03 cycles, r vector = 0.98, 5 experiments in this series and 4 additional experiments).

Thus the fast lumbar rhythm developed mainly in sacral and flexor-dominated segments of the lumbar cord, whereas the slow rhythm appeared in all lumbar segments, and it showed no flexor-extensor alternation. Recordings from the left and right L2 and S2 segments (Fig. 5) show that the fast rhythm exhibited an alternating left-right pattern and that the slow superimposed rhythm had a bilaterally synchronous pattern (Fig. 5B, 7/13 experiments, dL2–R = 0.998 ± 0.06 cycles, r vector = 0.93), an alternating left right pattern (Fig. 5D, 3/13 experiments, dL-R = 0.55 ± 0.18 cycles, r vector = 0.76) or a mixed and irregular (uniformly distributed, Rayleigh’s test) pattern (3/13 experiments, not shown). Transection of the spinal cord at the lumbosacral junction or at mid L5 blocked immediately the fast alternating lumbar rhythm without impairing the slow lumbar rhythm. The fast alternating-left-right rhythm of the sacral cord persisted under these conditions with
a robust alternating-left-right pattern. Analysis of eight transection experiments revealed that the cycle time of the $S_2$ rhythm was $2.7 \pm 0.8$ and $2.6 \pm 1.1$ s, and the left-right phase shift ($\phi_{LS2-RS2}$) was $0.5 \pm 0.07$ cycles, $r$ vector = 0.91, and $0.51 \pm 0.09$ cycles, $r$ vector = 0.86, before and after the transection, respectively.
To test whether the fast rhythm of the detached sacrococcygeal spinal segments (SC) and the slow rhythm of the detached thoracolumbar cord depended on activation of NMDA receptors, we transected the spinal cord at the lumbosacral junction in five different experiments and recorded the activity induced by methoxamine from L2 and S2 before and 30 min after addition of 200 µM methoxamine to the bath. The results of one of these experiments are shown in Fig. 5D. The slow lumbar rhythm (alternating left-right pattern in this case) could not be produced in the presence of 200 µM AP5 (top right), whereas a robust sacrococcygeal rhythm with a fast cycle time and an alternating-left-right pattern was initiated under these conditions [bottom right; cycle time = 2.5 ± 0.96 s (methoxamine) and 2.49 ± 0.88 s (AP5 and methoxamine), left-right phase shift φLS2-RS2 = 0.5 ± 0.07 cycles, r vector = 0.92, and 0.47 ± 0.1 cycles, r vector = 0.82, in the absence and presence of AP5, respectively]. The unchanged frequency of the fast SC rhythm in the presence of AP5 suggests that AP5 did not affect the SC CPGs and that the prolonged cycle time observed in preparations with attached SC segments in the presence of AP5 (Fig. 3) was probably due to changes in interactions between the TL and SC cords after the suppression of NMDA receptor-depended connectivity.

**FIG. 4.** The spatiotemporal pattern of the rhythm. Simultaneous recordings from the left L2–L5 and the left and right S2 ventral roots are shown immediately (A) and 10 min after (B, different experiment) addition of 100 µM methoxamine to the bath. Note the short synchronous rhythmic bursts in flexor-dominating segments and their absence in the extensor-dominated L5 in A and the appearance of a synchronous slow bursting in the left flexor- and extensor-dominating segments in B (↓). Frequency distribution of the cycle time of the methoxamine induced rhythm is shown in C. Data were pooled from a series of 5 experiments (slow and fast rhythm) and 9 additional experiments (slow rhythm).
As mentioned in the preceding text, the fast rhythm generated in the SC spinal segments appeared also in flexor- but not extensor-dominated segments of the lumbar cord. In a previous study, we have shown that changes in input resistance of motoneurons and their relation to efferent firing of the segmental ventral roots could be used to detect rhythmic changes in somatic inhibition produced by the sacrococcygeal rhythmogenic circuitry in neonatal rats (Lev-Tov et al. 2000). Therefore to infer about the organization of the pattern generating circuitry in the SC spinal cord.

**FIG. 5.** The pattern and segmental source of the α1-adrenoceptor-induced rhythms. A and B: recordings from the left and right L2 (DC) and S2 (100 Hz to 10 kHz) ventral roots are shown 5 min (A) and 15 min after (B) bath application of 100 μM methoxamine. C: after 60-min wash, the cord was transected at the lumbosacral junction and methoxamine has been added to the bath. Note that the fast lumbar rhythm and the slow sacral rhythm were blocked after the transection. Vertical bars are superimposed to demonstrate the alternating pattern of the fast rhythm. D: recordings of the slow and fast methoxamine-induced rhythms from the left and right L2 (DC) and S2 ventral roots (100 Hz to 10 kHz), are shown in the absence (left) and presence (right) of 200 μM AP5. Note that the slow lumbar rhythm was blocked and the fast sacral rhythm persisted in the presence of AP5. AP5 was added to the bath after 60 min of wash with Krebs saline; methoxamine (100 μM) was added 20 min after AP5.

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segments and the recruitment of flexor and extensor half centers in the lumbar cord, we used repetitive intracellular injection of hyperpolarizing current steps to examine changes in input resistance of motoneurons in S₂, the flexor-dominated L₂, and the extensor-dominated L₅ segments during the methoxamine induced rhythm. Our results are described in the following text:

Rhythmic fluctuations in input resistance of S₂ and lumbar flexor motoneurons

Figure 6A shows simultaneous ventral root recordings from S₂ and intracellular recordings from an S₂ motoneuron in a surgically detached SC cord in the presence of methoxamine (1 of 3 experiments performed in this series). A fast alternating

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FIG. 6. Crossed inhibition in sacral motoneurons during the fast methoxamine rhythm. A: intracellular recordings from a left S₂ motoneuron (L-S2MN) and extracellular recordings from the left and right S₂ ventral roots are shown in the presence of 100 µM methoxamine. - - - - , the pre-methoxamine resting membrane potential. B: recordings of the voltage transients produced in the same motoneurons by intracellular injection of −0.5 nA 100-ms current steps at 2 Hz. Motoneuron firing was blocked by injection of a continuous hyperpolarizing current (−0.75 nA) to the cell. Computer averaged records of the voltage transients produced during ipsi- and contralateral ventral root bursts are shown as insets (↓), the mean drop in $R_N$ during contralateral activity was 25±16%, P<0.001, (3 experiments). C: the amplitude of the voltage transients (multiplied by −1 for convenience, ⬤) and the left and right S₂ VRPs (---) recorded during the last 20 ms of each current step is plotted as a function of time (left). Cross-correlogram of the amplitude of voltage transients (LS2IC) vs. that of the contralateral VRPs (RS2) is shown superimposed with ±2 SE (- - - -) on the right. The drop in the amplitude of the voltage transients was in phase with contralateral VRPs.
rhythm with similar characteristics to the rhythm described in S₂ motoneurons of intact TL-SC preparations (e.g., Fig. 2) was produced under these conditions. The firing of the recorded motoneurons was blocked by injection of hyperpolarizing current (∼0.75 nA), and the voltage transients produced by repetitive injection of hyperpolarizing current steps (∼0.5 nA, 100 ms, 2 Hz) to the motoneuron were monitored. Continuous recordings of these voltage transients during the rhythm are shown with DC recordings of the left and right S₂ VRPs, in Fig. 6B. A substantial decrease in the amplitude of the voltage transients was evident during the troughs of the rhythmic oscillation in membrane potential. To analyze the relation between the voltage transients and the rhythmic activity, we averaged the voltage transients and the left and right S₂ VRPs produced during the last 20 ms of each current step and plotted the resultant mean amplitudes as a function of time in Fig. 6C.

The rhythmic variations in voltage transients and their relation to the contralateral ventral root potentials (VRPs) were examined using cross-correlation analysis (Fig. 6C, right). This analysis revealed significant rhythmic changes in the amplitude of the voltage transients (reflecting rhythmic changes in input resistance) and an inverse correlation between the amplitude of the transients and that of the contralateral VRPs, i.e., the drop in input resistance was in phase with contralateral activity.

Figure 7 shows recordings from L₂ motoneurons and the left and right L₂ ventral roots of a bilaterally intact (A, left) and a midsagittally split TL cords (A, right) during injection of hyperpolarizing current steps in the presence of methoxamine. The cross-correlation analyses of the relation between the amplitude of left and right L₂ VRPs and between the amplitude of the voltage transients and that of the contralateral VRPs under these conditions are shown in B (top and bottom correlograms, respectively). These data show that the rhythmic fluctuations in amplitude of the voltage transients observed in the bilaterally intact thoracolumbar cord were inversely correlated to the amplitude variations in the contralateral VRPs and that these oscillations in input resistance were not evident when the current steps were injected to L₂ motoneurons in the midsagittally split TL cord. Interestingly, the rhythmic activity in the midsagittally split TL cord exhibited a significant alternating-left-right pattern under these conditions. A similar pattern was found in three experiments in this series and four additional extracellular experiments. The relation between the amplitude of the voltage transients and contralateral ventral root activity was further examined using regression analyses of pooled standardized data (for details, see Fig. 7 legend). Summary plots of the standardized amplitude of the voltage transients versus that of the contralateral VRPs in the bilaterally intact (3 experiments) and midsagittally split (3 experiments) TL cord are shown with the linear regression lines in Fig. 7C (left and right, respectively). A significant negative slope (P < 0.0001) was found only for the bilaterally intact preparations [the slope and coefficient of determination (R²) were −0.56 ± 0.04 and 0.32 in the bilaterally intact and +0.09 ± 0.51 and 0.008, respectively, in the midsagittally split TL cords].

Lack of rhythmic fluctuation in input resistance of lumbar extensor motoneurons

Recordings from motoneurons in the extensor-dominated L₅ segments were performed in six different experiments to examine whether these motoneurons exhibit rhythmic inhibition during the activation of lumbar flexors. Figure 8 shows the voltage transients produced in a left L₅ motoneuron and the efferent activity recorded from the left and right L₅ and L₂ ventral roots in the presence of bath-applied methoxamine (A). The displayed records show no indication for systematic variations in input resistance of the recorded L₅ motoneuron during the rhythm. This notion is supported by the cross-correlation analysis of these data (B). A clear and significant alternating-left-right pattern of the L₂ rhythm is evident from the cross-correlogram of the amplitude of the left versus right L₂ VRPs (Fig. 8B, left). At the same time, there was no significant correlation between the amplitude of the voltage transients produced in the recorded L₅ motoneuron and the amplitude of the ipsilateral L₂ VRPs (B, right). Similar findings were observed in each of the experiments performed in this series. A summary plot of a regression analysis of the pooled standardized data obtained in these experiments is shown in Fig. 8C. The slope of the regression line between the amplitude of the voltage transients produced in L₅ motoneurons and the ipsilateral rhythmic activity −0.03 ± 0.024 was not significantly different from 0 (R² = 0.001 P < 0.15).

DISCUSSION

Initiation and modulation of rhythmic patterns by activation of adrenoceptors

The effects of NA and adrenoceptor agonists on rhythmogetic networks in the mammalian spinal cord have been described in detail in a number of preparations (vide ante). In contrast to the ability of adrenoceptor agonists to induce locomotion or locomotor like activity in spinal cats (for review see Rossignol 1998), bath application of NA to isolated spinal cords of neonatal rats has been shown to induce a slow rhythmic activity with irregular patterns in the lumbar (Kiehn et al. 1999; Sqalli-Houssaini and Cazalets 2000) and cervical cord (Morin et al. 2000). These effects of NA have been reported to be mimicked by bath applied α₁-adrenoceptor (Sqalli-Houssaini and Cazalets 2000; Morin et al. 2000) but not α₂- or β-adrenoceptor agonists (Sqalli-Houssaini and Cazalets 2000). In the present work, we showed that in addition to the slow and irregular rhythmic activity, bath-applied NA produced a fast alternating transient rhythm in the sacral (e.g., Gabbay et al. 2002) and rostral lumbar segments of the neonatal rat spinal cord. This rhythm has not been reported in previous studies (Kiehn et al. 1999; Sqalli-Houssaini and Cazalets 2000) probably due to its short-lasting nature and the appearance of the slow accompanying rhythm. We also showed that the fast rhythm could be induced and maintained for tens of minutes when the α₁-adrenoceptor agonist methoxamine was used instead of NA. Thus the transient nature of the fast NA-induced rhythm may be ascribed to activation of α₂- and/or β-adrenoceptors, which have been shown to slow down and inhibit the serotonin/NMDA-induced rhythm in the neonatal rat spinal cord (Sqalli-Houssaini and Cazalets 2000).

The slow and fast rhythms induced by α₁-adrenoceptor agonists differ not only in their cycle times but also in their segmental origin and the receptors involved in their generation. Our findings that the fast rhythm persisted in the SC spinal segments and was abolished in the TL segments after transecting the cord at the lumbosacral junction, and that the slow
rhythm continued in the detached TL and not the SC cord, suggested that the fast rhythm originates in the SC segments and the slow one is produced by the TL circuitry. The finding that the slow rhythm was blocked by the NMDA antagonist AP5, whereas the fast rhythm persisted in the presence of high concentrations of AP5, indicated that the slow rhythm reflects modulation of the NMDA-receptor-dependent rhythmicity of the TL cord similar to the suggestion of Kiehn et al. (1999), Sqalli-Houssaini and Cazalets (2000) and Morin et al. (2000), whereas the fast rhythm does not depend on NMDA receptors.
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and it may be actually initiated by activation of α1-adrenoceptors in the SC cord.

The reasons for the differential responsiveness of the TL and the SC central pattern generators (CPGs) to α1-adrenoceptor agonists are not known mainly due to lack of data concerning noradrenergic innervation of the tail moving networks in neonates. The differential responsiveness may arise from regional differences in the density and spatial distribution of adrenoceptors, from differences in the proportion of the known subtypes of adrenoceptors over constituents of the TL and SC CPGs, and from differential properties of the pattern generating circuitry of the TL and SC spinal segments. Further understanding of the ability of monoamines to induce NMDA-independent rhythmic patterns in the isolated SC and not in the isolated TL spinal segments should await additional physiological and histochemical studies of noradrenergic innervation of these regions.

The mechanisms involved in the generation of the fast rhythm by NA are not known. NA has been shown to decrease the potassium conductance and the input resistance of neonatal rat motoneurons and to depolarize them and increase their excitability (Morin et al. 2000; Parkis et al. 1995; White et al. 1991). These effects of NA were mimicked by α1-adrenoceptor agonists (Morin et al. 2000; Parkis et al. 1995). In adult motoneurons, NA has been shown to increase the voltage-dependent persistent inward currents (I\textsubscript{PSC}) (Lee and Heckman 1999) involved in generation of plateau potentials that are capable of producing a prolonged neuronal discharge in response to a transient activation and thereby shape the motor output (for review, see Perrier et al. 2002). Plateau potentials have been described in various spinal neurons, including putative pattern generating interneurons of the neonatal rat spinal cord (Kiehn et al. 1996), and their possible activation in the presence of NA may contribute to rhythmogenesis. Our attempts to detect plateau potentials in P\textsubscript{2}-P\textsubscript{4} spinal motoneurons in the presence of NA and to affect the SC rhythm by application of nifedipine, a blocker of the L-type calcium channel whose activation is a major source of I\textsubscript{PSC} (Hounsgraad and Kiehn, 1989; Russo and Hounsgraard 1996; for review, see Perrier et al. 2002), have not been successful (A. Medini and A. Lev-Tov unpublished results), possibly due to the immature nature of the L-type channel at this postnatal age (Jiang et al. 1999b). Additional studies in this regard are currently performed in our laboratory.

Source of the fast rhythmic drive potential

As mentioned in the preceding text, the fast lumbar rhythm is significantly delayed comparing to the SC rhythm, and it depends on the presence of SC spinal segments (e.g., Fig. 4). An important question is whether its generation involves activation of the TL locomotor pattern generators by SC projections. The pattern of the fast lumbar rhythm is different from that of the locomotor rhythm. The locomotor rhythm is characterized by alternating flexor-extensor and by alternating-left-right patterns, while the fast lumbar rhythm is manifested as an alternating activation of the flexor-dominating segments of the cord.

Generally speaking a hypothetical agonistic half-center can be activated separately from its antagonistic half-center (Hochman and Schmidt 1998). If this was the case, the anticipated rhythmic drive potential of lumbar flexor motoneurons would be either composed of phasic-excitation alone (see Hochman and Schmidt 1998) or phasic excitation alternating with phasic inhibition (originating from the contralateral half-center) (see Kjaerulf and Kiehn 1997). In either case, one would expect evidence of phasic inhibition of the extensor motoneurons (e.g., in L\textsubscript{5}). However, intracellular recordings of extensor-dominated L\textsubscript{5} motoneurons and injection of current steps into these motoneurons revealed no rhythmic changes in their input resistance in the presence of methoxamine (Fig. 8). This finding suggests a lack of rhythmic inhibitory input in L\textsubscript{5} motoneurons from components of an ipsilateral half-center as well as an absence of crossed inhibitory input.

In addition, a substantial decrease in input resistance of both sacral (Fig. 6) and the flexor-dominated L\textsubscript{2} motoneurons (Fig. 7) was detected during the troughs of the rhythmic drive potential induced by methoxamine. This rhythmic inhibition of L\textsubscript{2} motoneurons coincided with the contralateral ventral root firing and was abolished in the midsagittally split TL cord. This suggests it was due to activation of crossed inhibitory pathways as has been proposed for the sacral rhythm produced by repetitive stimulation of sacrocaudal afferents (Lev-Tov et al. 2000). Interestingly, the alternating pattern of the fast lumbar rhythm could be maintained for many minutes in the absence of the phasic inhibitory drive in the midsagittally split thoracolumbar cord (Fig. 7). These observations suggest that the purely excitatory rhythmic drive potential observed in L\textsubscript{2} motoneurons of midsagittally split thoracolumbar segments originates from ascending/propriospinal projections of the ipsilateral sacral cord, while the left-right phase is maintained under these conditions by crossed inhibitory pathways between the left and right sacral pattern generators. Given that axons of commissural neurons cross the midline at the segmental level of their somata (Eide et al. 1999; Stokke et al. 2002), this suggestion implies that the crossed inhibition found in rostral lumbar motoneurons of bilaterally intact TL cords during the rhythm is not due to axons from the sacrocaudal inhibitory interneurons that project rostrally and cross at the lumbar cord.
FIG. 8. The \( R_n \) of lumbar extensor motoneurons does not vary with the fast rhythm. The voltage transients produced by repetitive (2 Hz) intracellular injection of current steps (−0.5 nA, 100 ms) to a left L₅ motoneuron (L-L₅MN) and left (L) and right (R) ventral root recordings of L₂ (A) are shown with cross-correlograms of the amplitude of the left and right L₂ VRPs (B, left) and the amplitude of the voltage transients (LL₅IC) vs. the ipsilateral L₂ VRPs (L-L₂, B, right). top and bottom - - - denote ±2 SE.

The L₂ recordings (top) were rectified and low-pass filtered at 50 Hz. The insets (⊥) are computer-averaged records sampled during ipsi-and contralateral L₂ bursting, respectively. Summary plot of the normalized amplitude of the voltage transients produced in L₅ motoneurons vs. the amplitude of the ipsilateral L₂ and S₂ VRPs (the activity of the ipsilateral L₂ and S₂ was highly synchronized (in phase) during the fast rhythm, see Figs. 1, 3, 4, and 5) is shown in C. The plot is based on standardized data (see Fig. 7C) pooled from 6 experiments (L₂ and S₂ VRPs were recorded in 2 and 4 of the experiments, respectively). Regression analysis of these data revealed a slope that was not significantly different from 0, see text.
The crossed inhibitory pathways between the half centers include direct projections to contralateral motoneurons. These projections account for the rhythmic drop in \( R_p \) of sacral motoneurons during the fast methoxamine rhythm. Excitatory projections (ascending/propriospinal) of the sacral half centers to lumbar-flexor motoneurons and to crossed inhibitory lumbar interneurons produce alternation of phasic excitation and inhibition in the rhythmic drive potential of bilaterally intact \( L_2 \) motoneurons. After a thoracolumbar midline split, the phasic inhibition onto \( L_2 \) motoneurons is abolished and the alternating pattern of the split lumbar cord is maintained by the sacral crossed-connectivity. But rather due to lumbar commissural interneurons activated by ascending/propriospinal excitatory projections from the sacral cord. Schematic representation of this hypothesis is illustrated in Fig. 9.

**Functional implications**

What is the functional significance of the fast rhythm? As mentioned in the preceding text, the fast rhythm was generated primarily in the SC spinal segments by adrenoceptors activation, and it produces rhythmic tail movements and rhythmic activation of the regional axial musculature. In this way, descending noradrenergic projections onto the sacroccocygeal cord may be able to control tail movements during different behavioral repertoires. Moreover, in the present study, we showed that the fast rhythm appeared also in flexor-dominating segments of the lumbar cord. Thus the activated SC circuitry is capable of increasing the probability of recruiting lumbar flexors, a feature that may be used to facilitate the combined appearance of rhythmic tail movements and locomotor activity in response to stimulation of sacrocaudal dermatomes (Bonnot et al. 2002a; Delvolvé et al. 2001; Lev-Tov and Delvolvé 2000; Lev-Tov et al. 2000; Smith et al. 1988; Strauss and Lev-Tov 2003; Whelan et al. 2000) during nocifensive/escape type of behavior.

Finally, the ability of the NMDA-serotonin-dependent rhythmicity of the TL pattern generators to drive the SC networks in the presence or absence of NA (Cazalets and Bertrand 2000; Gabbay et al. 2002; Kremer and Lev-Tov 1997) may be used to produce coordinated limb and tail movements with the required frequency during swimming (Cook et al. 2001; Gabbay et al. 2002), climbing, and turning (Bennett et al. 1999; Wada and Shikaki 1999; Walker et al. 1998).

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