Monoaminergic Control of Cauda-Equina-Evoked Locomotion in the Neonatal Mouse Spinal Cord

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Gordon, Ian T. and Patrick J. Whelan. Monoaminergic control of cauda-equina-evoked locomotion in the neonatal mouse spinal cord. J Neurophysiol 96: 3122–3129, 2006. First published September 6, 2006; doi:10.1152/jn.00606.2006. Monoaminergic projections are among the first supraspinal inputs to innervate spinal networks. Little is known regarding the role of monoamines in modulating ongoing locomotor patterns evoked by endogenous release of neurotransmitters. Here we activate a locomotor-like rhythm by electrical stimulation of afferents and then test the modulatory effects of monoamines on the frequency, pattern, and quality of the rhythm. Stimulation of the cauda equina induced a rhythm consisting of left-right and ipsilateral alternation indicative of locomotor-like activity. First, we examined the effects of noradrenaline (NA), serotonin (5-HT), or dopamine (DA) at dose levels that did not elicit locomotor activity. Bath application of NA and DA resulted in a depression of the cauda-equina-evoked rhythm. Conversely, bath-applied 5-HT increased both the amplitude and cycle period of the evoked rhythm, an effect that was mimicked by the addition of 5-HT2 agonists to the bath. Application of 5-HT2 agonists disrupted the evoked behavioral rhythm. Next, we examined the effects of NA α1 and α2 agonists and found that the suppressive effects of NA on the rhythm could be reproduced by adding the α2 agonist, clonidine, to the bath. In contrast, bath application of the α1 agonist, phenylephrine, increased the amplitude and duration of the cycle period. Finally, the suppressive effects of DA were not replicated by the administration of D1, D2, or D3 agonists although application of NA α2 agonists reversed the effects of DA. Application of D1 agonists, increased the amplitude of the bursts but did not affect the cycle period. Our results indicate that monoamines can control the expression, pattern, and timing of cauda-equina-evoked locomotor patterns in developing mice.

INTRODUCTION

Locomotion in humans and many mammals develops in the weeks to months after birth. The postnatal period is the critical time when descending projections from the brain mature and modulate spinal circuits that generate locomotion (Vinay et al. 2002). As early as birth, tail-pinching evoked stepping behavior appears to be tightly controlled by descending projections in neonatal rats. After a complete transection of the thoracic spinal cord, a tail pinch can evoke longer bouts of locomotor behavior (Norreel et al. 2003). This suggests that descending bulbo-spinal or propriospinal projections can functionally regulate afferent transmission onto spinal pattern-generating circuits. The monoamines serotonin (5-HT), noradrenaline (NA), and dopamine (DA) are likely candidates for modulating the expression of this air-stepping behavior. Monoaminergic projections are among the first supraspinal inputs to reach the spinal cord where they modulate and influence the development of spinal reflexes and spinal locomotor circuits (Allain et al. 2005; Ballion et al. 2002; Branchereau et al. 2002; Jordan and Schmidt 2002; Kiehn et al. 1999; Liu and Jordan 2005; Schmidt and Jordan 2000; Sillar et al. 1998; Sqalli-Houssaini and Cazalets 2000).

A great deal of insight regarding the rhythmogenic and modulatory capability of monoamines has been generated by bath application of drugs in experiments using neonatal preparations. However, the interpretation of the modulatory capabilities of these drugs is complicated by the fact that monoamines are often used to elicit a baseline rhythm. This rhythm tends to be slow, and it is unclear how physiologically relevant it may be. Recent studies demonstrate that activation of sacral afferents in vitro can readily evoke rhythmic locomotor-like activity in neonatal mouse or rat spinal cord preparations (Delvolve et al. 2001; Gabbay et al. 2002; Lev-Tov and Delvolve 2000; Lev-Tov et al. 2000; Strauss and Lev-Tov 2003; Whelan et al. 2000). It is likely that this system is the in vitro analog (Lev-Tov et al. 2000; Whelan et al. 2000) of escape-like locomotor behavior that can be elicited from neonatal pups in vivo by pinching the tail (Norreel et al. 2003). Electrical stimulation of cauda equina afferents is advantageous for producing locomotor activity because the basic circuitry necessary for producing this behavior is contained within the spinal cord (Strauss and Lev-Tov 2003). Another advantage is that because descending projections are cut, there is likely little to no monoaminergic modulation of this self-contained circuit. Finally, the cycle period of the rhythm is ~1 s, which is close to the observed behavior in vivo (Cheng and P. J. Whelan, unpublished observations). Thus cauda-equina-evoked rhythms offer an excellent base rhythm to examine the effects of monoaminergic modulation.

Here we test the hypothesis that monoamines can modulate rhythms evoked by cauda equina stimulation in the neonatal mouse. Our data suggest that even at an early stage of development, monoamines can effectively modulate escape-like responses in a physiologically appropriate frequency range. A portion of this work has been published in abstract form (Gordon and Whelan 2004).

METHODS

Experiments were performed on Swiss Webster mice (Charles River Laboratories) 1–2 days old (P1–P2, n = 76). The animals were...
anesthetized by hypothermia, decapitated, and eviscerated using procedures approved by the University of Calgary Animal Care Committee. The remaining tissue was placed in a dissection chamber filled with oxygenated (95% O2–5% CO2) artificial cerebrospinal fluid (ACSF; concentrations in mM: 128 NaCl, 4 KCl, 1.5 CaCl2, 1 MgSO4, 0.5 NaH2PO4, 21 NaHCO3, and 30 d-glucose). A ventral laminaectomy exposed the spinal cord sparing as much of the cauda equina as possible, and the ventral and dorsal roots were cut. The cord was transected at the caudal end of the cervical enlargement (C8–T1) and then was gently removed from the vertebral column. The isolated spinal cord was allowed to recover for ≥30 min before being transferred to the recording chamber and superfused with oxygenated (95% O2–5% CO2) ACSF. The bath solution was heated gradually from room temperature to 27°C. Then the preparation was allowed to equilibrate for ≥30 min.

Electrophysiological recordings and activation of locomotor networks

Neurograms were recorded with suction electrodes into which segmental ventral roots were drawn. Generally, neurograms were recorded from the following ventral roots: the left and right lumbar 2 (L2) ventral roots, an L5 ventral root, and in select experiments, a sacral 4 (S4) root as well. The neurograms were amplified (100–20,000 times), filtered (100 Hz to 1 kHz or DC–1 kHz), and digitized (Axon Digidata 1322A) for future analysis. Alternating segmental and ipsilateral ventral root bursting patterns were taken to be indicative of fictive locomotion (Whelan et al., 2000).

Constant current stimulus trains (A360 World Precision Instruments, AMPI Master 8 pulse generator) were delivered to coccygeal roots via a suction electrode. To determine the stimulus threshold (T), single pulses were delivered at increasing intensities until a polysynaptic reflex response was elicited (2–350 μA). Pulses were delivered every three minutes (4 Hz, 40 pulses, T-3T range) at a constant intensity throughout an experiment (Whelan et al., 2000).

After recording 15 min of control rhythms, monoaminergic agonists or antagonists were added to the recirculating ACSF, and the rhythm was recorded for another 45 min. Finally, between 500 and 1,000 mL of preheated and oxygenated ACSF was washed through the system, and 30 min of washout sweeps were recorded. Temperature data from the bath were stored in a separate channel during sweep acquisition.

Pharmacology

These experiments utilized a variety of monoaminergic agonists and antagonists. Agonist concentrations were established using previous reports and by testing in preliminary experiments whether the effects were reversible using appropriate antagonists. These drugs included: NA (4 μM, Sigma-Aldrich), DA (10–20 μM, Sigma-Aldrich), 5-HT (10 μM, Sigma-Aldrich), phenylephrine, an α1 adrenergic receptor agonist (25 μM, Sigma-Aldrich), clonidine, an α2 adrenergic agonist (0.5 μM, Sigma-Aldrich), SKF-81297, a D1 dopaminergic receptor agonist (10 μM, Sigma-Aldrich), LE300, a D2 dopaminergic receptor antagonist (1 μM, Tocris), quinpirole, a D2 dopaminergic receptor agonist (10–40 μM, Sigma-Aldrich), PD-128907, a D3 dopaminergic receptor agonist (10 μM, Tocris), 5-CT, a 5-HT1D, serotonergic receptor agonist (1–10 μM, Sigma-Aldrich), WAY-100635, a 5-HT1A, serotonergic receptor antagonist (1–5 μM, Sigma-Aldrich), GR 127935 hydrochloride, a 5-HT2A serotoninergic receptor antagonist (0.1–1 μM, Sigma-Aldrich), and α-methyl-5-HT, a 5-HT2, serotonergic receptor agonist (2 μM, Sigma-Aldrich). We checked for selectivity of the agonists in preliminary experiments using ketanserin, a 5-HT1A antagonist (4 μM, Sigma-Aldrich), SB-269970, a 5-HT7, serotonergic receptor antagonist (5 μM, Tocris), prazosin, an α1 adrenergic receptor antagonist (2 μM, Sigma-Aldrich), and yohimbine, an α2 adrenergic receptor antagonist (2 μM, United States Biochemical). Fusaric acid (100 μM, Sigma-Aldrich) was also used to block dopamine-β-hydroxylase activity in select experiments.

Data analyses

Data were analyzed using custom written programs (MatLab, MathWorks, Natick, MA) as well as commercially available programs (Statistica, StatSoft, Tulsa, OK). The section of data analyzed for rhythmicity was defined as the duration of the stimulus train for cauda equina experiments. The data were digitally high pass filtered at 100 Hz, rectified, and then low-pass filtered before being reduced by a factor of 10. A cospectral density analysis comparing the segmental L2 roots was performed using Statistica. The cospectral density and primary frequency were determined as the maximum value of the cospectral density of the compared neurograms. Phase was calculated by averaging the phase shift values for the frequencies that represented the primary 75% of the component frequencies (Strauss and Lev-Tov 2003). Phase and period values were not compared when the cospectral density was reduced <10% of control. To measure the average long-latency polysynaptic potential (LLPP) elicited from cauda equina stimulation, we calculated the mean amplitude for a 10- to 200-ms window after the stimulus initiation and subtracted an average baseline value from 200 ms of data just prior to stimulation. When modulatory effects on the rhythm were recorded, we averaged bursts together to examine mean changes on the burst amplitude and duration. To improve the clarity of the traces, we removed the stimulus artifacts.

Statistics

For reporting purposes, cospectral density, and LLPP data were normalized to control values set to the arbitrary value of 100%. Subsequent trial conditions were compared with control using a one-tailed t-test and significance was set at P < 0.05 (Statistica). Phase values and cycle periods were not normalized. For multiple comparisons, we used a repeated-measures one-way ANOVA followed by a Tukey post hoc test to detect significant differences where P < 0.05 (SigmaStat). For some data, we examined the correlation between the LLPP and the change in cycle period. Goodness of fit was calculated to determine if linear regression was suitable. To determine whether the correlation was significant we tested whether the slope of the linear regression line was greater than zero (GraphPad Prism).

RESULTS

Similar to previous reports, stimulating cauda equina afferents with a 4-Hz, 10-s train every 3 min induced robust rhythmic activity in isolated spinal cord preparations (Lev-Tov et al. 2000; Whelan et al. 2000). Alternate bursting of segmental L2 roots and ipsilateral L2–L5 roots were taken to indicate locomotor-like activity (Bonnot et al. 2002; Whelan et al. 2000). To determine the stability of the evoked rhythm, we ran control experiments in which C8-cauda equina spinal cords were stimulated every 3 min for a minimum of 90 min. The rhythm was assessed for the stability of the primary cospectal density, phase and frequency, and the amplitude of the long latency polysynaptic potential (LLPP) was measured (Fig. 1). All of these values remained stable >90 min.

Bath application of monoamines modulates afferent-evoked locomotion

To determine the effect of monoaminergic neurotransmitters on the neural circuits that mediate afferent-evoked locomotion, we bath applied each of the monoamines at concentrations lower than those previously found to induce pharmacological locomotor patterns (Kiehn et al. 1999; Madriaga et al. 2004; Whelan et al. 2000). When DA (10–20 μM) was bath applied,
the rhythm was completely blocked as revealed by the significant reduction in the cospectral density (n = 5, P < 0.05). Similarly, the addition of NA (4 μM) also blocked the afferent-evoked pattern (n = 5, P < 0.05). Phase and cycle period values are not reported for DA or NA as the cospectral density was reduced <10% of control (Fig. 2). Although NA or DA blocked the rhythm, baseline neurogram activity from all roots increased and sporadic bouts of rhythmic bursting were found to persist in between stimulus trains. The application of 5-HT (10 μM) did not reduce the cospectral density (n = 5, P > 0.1). However, the burst duration did increase (data not shown), and the cycle period was also significantly increased (n = 5, P < 0.01, Fig. 2), whereas the phase was unaffected (n = 5, P > 0.1, data not shown). Like NA and DA, 5-HT increased the baseline activity for all recorded neurograms (data not shown). The average initial polysynaptic potentials in the right and left L2 as well as the L5 neurograms were significantly decreased with the addition of 5-HT (10 μM, n = 5, P < 0.001), DA (10–20 μM, n = 5, P < 0.05), or NA (4 μM, n = 5, P < 0.001, Fig. 3). This inhibition was reversed for all roots recorded following washout (n = 5, P > 0.1).

The time course of activation for serotonin, dopamine and noradrenaline were all similar, exerting their effects in <6 min. It has been reported that the effects of dopamine on spinal circuitry can last for an hour after washout (Barriere et al. 2004). However, in our study, the effects of dopamine were not long-lasting, and generally we saw a return to control conditions within the first half hour of wash.

Activation of 5-HT₂ receptors mimics the modulatory effect of 5-HT

Next we tested whether activation of specific classes of serotonergic receptors with bath-applied agonists could replicate the effects of 5-HT (Fig. 4). Addition of the 5-HT₂ A i. Control ii. Control iii. Control

FIG. 2. Serotonin (5-HT), noradrenaline (NA), or dopamine (DA) differentially affect afferent-evoked locomotion. A: representative traces illustrating the effect of bath applying 10 μM 5-HT (i), 4 μM NA (ii), or 20 μM DA (iii). The duration of the 10-s stimulus train is depicted by horizontal lines below the traces. B: graph demonstrating the normalized cospectral density values. C: graph showing the associated cycle period of the evoked rhythms. X: trials where the cospectral density was reduced <10% of control values. *, significant difference from control (P < 0.05). Error bars represent the SE.
Activation of α2 receptors mimics the inhibitory effect of NA

To investigate which receptors may be underlying the inhibitory actions of NA, we bath applied adrenergic α2 and α1 receptor agonists (Fig. 5). First, we utilized the α1 agonist phenylephrine (25 μM) and found that although it did not decrease the cospectral density (n = 5, P > 0.1) or the phase (n = 5, P > 0.1). However, both the cycle period (n = 5, P < 0.01, Fig. 4) and burst duration were increased (supplementary Fig. 1B). Next we activated the 5-HT7 receptors in isolation by applying WAY-100635, a 5-HT1A receptor antagonist (1 μM), and GR 127935 hydrochloride, a 5-HT1B receptor antagonist (1 μM), in combination with 5-CT (1 μM). This resulted in a significant destabilization of rhythmicity as measured by the cospectral density (n = 5, P < 0.05). Neither the phase nor the period (n = 5, P > 0.1) was significantly affected (Fig. 4). Finally, the LLPP was significantly reduced following activation of either the 5-HT2 (n = 5, P < 0.05) or the 5-HT7 receptors (n = 5, P < 0.01, Fig. 4). We did not observe a correlation between the reduction in the LLPP by 5-HT2 and the modulation of the cycle period (P > 0.1; see supplementary Fig. 2A).

Activation of none D1, D2, or D3 receptors mimics the inhibitory effect of DA

In the next series of experiments, we examined the ability of DA receptor agonists to mimic the inhibitory action of DA. When applied at 20 μM, the D1 agonist, SKF-81297, did not result in a decreased cospectral density between the segmental L2 neurograms. In fact, SKF-81297 stabilized the alternating rhythm, an effect similar to that found on drug-induced rhythms (Madriaga et al. 2004) as shown by the significant increase in cospectral density (n = 7, P < 0.05, Fig. 6B). Quinpirole, the D2 agonist (10–40 μM), also did not mimic DA’s action and produced no effect on the cospectral density (at 40 μM, n = 5, P > 0.01, Fig. 6B). Conversely, both the D1 and D2 agonists decreased the LLPP significantly (n = 7 and n = 5, respectively, P < 0.05, Fig. 6D). Finally, the D3 agonist, PD-128907 (10 μM), failed to decrease the cospectral density (n = 5, P > 0.1, Fig. 6A) or the LLPP (n = 5, P > 0.1, Fig. 6D). The cycle period was unaffected by the addition of D1 or D3 agonists (n = 5–7, P > 0.1, Fig. 6C); however, the addition of quinpirole, the D2 agonist did significantly increase the cycle period (n = 5, P < 0.05). Phase values also remained constant following D1, D2, or D3 agonist addition (n = 5–7, P > 0.1). SKF-81297 did increase the amplitude of averaged bursts (see supplementary Fig. 1A), likely contributing to part of the increase in the cospectral density.
Dopamine acts directly on adrenergic receptors to inhibit rhythmicity

The D2 or D3 agonists did not suppress rhythmicity, and in fact, D1 agonists may have increased the stability of the rhythm and amplitude of the bursts. However, when bath applied at 10–20 μM, DA completely inhibits the afferent-evoked rhythm (Fig. 2Aiii). Therefore to determine if DA was being converted to NA, which was then activating α2 receptors to suppress the rhythm, we conducted a series of experiments in which the dopamine-β-hydroxylase inhibitor fusaric acid (100 μM) was added to the bath. After recording five control sweeps, we added 20 μM DA as previously described and found that DA was still capable of inhibiting the rhythm as measured by the cospectral density (n = 4, P < 0.01, data not shown). Consequently, the dopaminergic effect was not primarily mediated by a conversion of DA to NA. Finally, previous results have shown that DA is capable of binding to α2 receptors directly in the preoptic area of the rat (Cornil et al. 2002). Thus we tested whether this cross-reaction was also occurring in our preparation. Addition of the α2 adrenergic antagonist yohimbine (2 μM) after DA (10–20 μM) reversed the dopaminergic effect, allowing the locomotor rhythm to recover to control levels (n = 5, P > 0.1, supplementary Fig. 3). The addition of yohimbine without previous administration of DA did not affect the rhythm (cospectral density, n = 5, P > 0.1). Initially, we hypothesized that the addition of yohimbine after DA would lead to an increase in the cospectral density above control levels due to an unmasking of DA’s effect on D1 receptors. Therefore our protocol included the application of the D1 antagonist LE300 (1 μM) to reverse the predicted effect. However, although we observed a trend for an increase, this was not significant, and so it is not surprising that bath application of LE300 did not reduce the cospectral density when compared with the yohimbine trials (n = 5, P > 0.1).

DISCUSSION

In neonatal animals, monoaminergic modulation of spinal CPGs has been mainly tested using pharmacologically evoked rhythms. Accordingly, there is a lack of data regarding the monoaminergic modulation of CPGs activated by endogenous release of neurotransmitters. The data from this study indicate that monoamines exert receptor dependent, modulatory effects on the pattern and frequency of a rhythm evoked by cauda equina stimulation.

Monoaminergic depression of rhythmic activity

Activation of lumbar networks by cauda equina stimulation depends on relay propriospinal interneurons within the sacral spinal cord (Strauss and Lev-Tov 2003). Presumably, one site of action of NA, DA, and 5-HT would be on the first-order

FIG. 5. α adrenergic receptor agonists can modulate afferent-evoked rhythmicity. A: representative traces illustrating the effect of α1 (phenylephrine, 25 μM) and α2 (clonidine, 0.5 μM) receptor activation. —, duration of the 10-s stimulus train. B: graph demonstrating the cospectral density values. C: graph showing the associated cycle period of the evoked rhythms. D: graphical representation of the normalized averaged LLPP values. *, significant difference from control (P < 0.05). Error bars represent SE.
interneurons within the dorsal horn of the sacral cord (Millan 2002). The suppression of the afferent-evoked rhythm and polysynaptic reflexes by clonidine, which mimicked the effects of NA, are consistent with the powerful inhibitory effects of α2 agonists on afferent transmission (Millan 2002) and with the high α2 receptor expression in the superficial dorsal horn, lamina X, and dorsal laminae (Shi et al. 1999; Stone et al. 1998).

Because DA potently blocked cauda equina evoked locomotor activity, it was surprising that none of D1, D2, or D3 receptor agonists could block rhythmicity. This was in spite of the fact that D1 and D2 receptor agonists reduced evoked polysynaptic potentials as predicted by previous reports (Clemens and Hochman 2004). This suggests that transmission from propriospinal relay interneurons in the sacral cord to the lumbar CPG was not affected. Although surprising, these data are consistent with reports demonstrating differential control of nociceptive and group II reflexes by monoamines in adult animals (Jankowska 2001; Jankowska and Hammar 2002; Millan 2002). Our data suggest that DA inhibition of the evoked rhythm is mediated through activation of α2 receptors. These effects were not due to conversion to NA because blocking dopamine-β-hydroxylase did not block DA-mediated inhibition. DA has been observed to cross react with α2 receptors in other parts of the brain (Cornil et al. 2002), and paracrine release of DA within the dorsal horn could potentially activate neighboring synapses (Mundorf et al. 2001; Ridet et al. 1993). One issue is that our DA bath concentrations were high, and concentration-dependent effects on evoked reflexes have been reported in the 10- to 20-μM range using a mouse spinal cord preparation (Clemens and Hochman 2004). Nevertheless, it does not detract from our observations that direct activation of DA receptors does not suppress cauda equina evoked locomotor-like activity.

5-HT is known to decrease polysynaptic reflexes, and our data confirm these findings in the mouse (Wallis et al. 1993a,b; Yomono et al. 1992). Although 5-HT suppressed polysynaptic potentials, we did not observe a disruption of rhythm activity. 5-HT is known to affect afferent transmission, and the lack of an large inhibitory effect is most likely because 5-HT activates ≥15 receptor subtypes, many of which contribute to network excitation as described in the following text.

**Monoaminergic modulation of rhythmic activity**

Although bath application of NA and DA blocked the cauda-equina-evoked rhythm, both NA and DA modulate bath-evoked rhythms in neonates (Barriere et al. 2004; Kiehn et al. 1999; Madriaga et al. 2004; Sqalli-Houssaini and Cazalets 2000; Whelan et al. 2000), and it is well known that the precursor L-DOPA facilitates locomotor networks (Jankowska et al. 1967a,b; Tucker and Stetrouwer 2000). When we used agonists for monoaminergic receptors that are preferentially expressed in the ventral horn, the rhythm slowed down, suggesting a site of action within the CPG. Activation of either α1 or 5-HT2 receptors significantly decreased the frequency of the evoked rhythm and increased the duration of the bursts. Even though the rhythm slowed compared with control conditions, the frequency was within a physiologically normal range.

Despite having similar effects on the frequency, 5-HT2 agonists increased the quality of the L5 pattern to a greater extent compared with α1 agonists. This agrees with previous electrophysiological and immunocytochemical data showing uniform rostrocaudal expression of 5-HT2 receptors in the lumbosacral segments (Liu and Jordan 2005; Schmidt and Jordan 2000) and early expression within the embryonic spinal cord of mice (Lauder et al. 2000). The modulatory actions of 5-HT2 receptors on CPG frequency and burst duration are consistent with high expression in the ventral horn (Bursa et al. 2001; Fonseca et al. 2001; Schmidt and Jordan 2000), and 5-HT can alter the excitability of commissural interneurons located in the ventral horn (Zhong et al. 2006a,b). On the basis of previous reports, we were expecting 5-HT7 agonists to potentiate and modulate the rhythm (Liu and Jordan 2005; Madriaga et al. 2004; Pearlstein et al. 2005). This was not the case, and instead the rhythm was reversibly weakened after addition of 5-HT7 agonists. Possible explanations include actions at the dorsal horn where 5-HT7 receptors are known to be clustered in addition to the ventral horn, which could explain the concomitant reduction in polysynaptic potentials (Cina and Hochman 1998; Doly et al. 2005; Schmidt and Jordan 2000).

Our data that show α1 agonists slowing the cauda-equina-evoked locomotor rhythm and increasing burst duration also suggests a site of action within the CPG. The data are comparable to immunocytochemical and electrophysiological data alluding to a locus of action within the ventral horn (Jones et al. 1985; Sqalli-Houssaini and Cazalets 2000). One notable difference is that our work demonstrates that α1 agonists slow the cauda-equina-evoked rhythm down, whereas the opposite result was obtained using pharmacologically evoked rhythms in the neonatal rat (Sqalli-Houssaini and Cazalets 2000). Modeling studies illustrate that increases in inhibitory commissural drive slow the rhythm down, whereas decreases in inhibitory drive can increase the strength of bursts as well as the frequency of the rhythm (Dale 1995; Hellgren et al. 1992). However, this may not be the only possibility because recent data show that inhibition of inhibitory ipsilateral V1 interneurons slow down the rhythm (Gosgnach et al. 2006).

Interestingly, both α1 and 5-HT2 agonists inhibited the amplitude of polysynaptic reflexes recorded from both L2 and L5 neurograms. Other reports have showed inhibition of spinal reflexes by 5-HT2 in neonatal rats (Yomono et al. 1992), and high expression of 5-HT2 receptors have been reported in lamina II of rats (Doly et al. 2004). The inhibition of afferent transmission may be due to potentiation of glycine conductances in first-order dorsal horn neurons by activation of inhibitory interneurons (Li et al. 2002). For both 5-HT2 and α1 agonists, we observed no correlation between the depth of the polysynaptic reflex modulation and the modulation of network frequency and burst duration. This suggests a complex control of polysynaptic reflexes and networks at birth similar to the proposed role for monoamines in adults (Bannatyne et al. 2003; Hammar and Jankowska 2003; Hammar et al. 2004; Jacobs and Fornal 1993; Jankowska and Hammar 2002; Jankowska et al. 2005).

In contrast to the effects of 5-HT2 or α1 agonists, D1 agonists increased the amplitude of the burst but did not affect the timing of the rhythm. Qualitatively similar effects were observed when DA was applied in the presence of α2 antagonists. These results are consistent with reports of high levels of D1 immunoreactive receptors in ventral horn neurons (Dubois et
al. 1986; Holstege et al. 1996; Weil-Fugazza and Godefroy 1993) and with electrophysiological data that demonstrate that DA increases ventral neuronal excitability (Barasi and Roberts 1977; Smith et al. 1995). However, our data suggest that D1 actions are mainly on motoneurons and not on the network, which is contrary to reports that demonstrate that D1 agonists either alone or in combination with 5-HT can evoke and modulate rhythms in the rat and mouse (Barriere et al. 2004; Madriaga et al. 2004; Whelan et al. 2000). One explanation could be the differences in the drug concentrations used or the different methods used to evoke locomotor activity.

Functional considerations

Several lines of evidence suggest monoamines could produce similar effects in the intact neonate. 5-HT and NA fibers exist at all spinal segments in the perinatal rodent (Ballion et al. 2002; Rajaoefetra et al. 1992). At least a portion of these fibers are likely functional because stimulation of the parapyramidal area in the brain stem elicits 5-HT-dependent locomotor-like activity (Liu and Jordan 2005) and separate studies confirm the release of 5-HT and NA metabolites in the ventral horn of adult and neonatal rats during brain stem stimulation or exercise (Gerin et al. 1994, 1995; Jordan and Schmidt 2002). Less data are available for the dopaminergic system; however, DA fibers and receptors are present in the rat spinal cord (Holstege et al. 1996; Ridet et al. 1992; Yoshida and Tanaka 1988) and are functional (Fleetwood-Walker et al. 1988). The extent to which DA, 5-HT, or NA contributes to reflex and network modulation in the intact neonate is unknown. However, our data allude to monoamines exerting a complex set of actions on the expression of escape-like behavior as early as birth. Taken together, this suggests that the descending control of reflex pathways onto motor circuits may be well developed at early stages of development. It will be interesting to test this hypothesis using new strains of mice where classes of interneurons forming reflex and locomotor spinal circuits can be identified (Gordon and Whelan 2006).

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