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Modulation of Locomotor Activity by Multiple 5-HT and Dopaminergic Receptor Subtypes in the Neonatal Mouse Spinal Cord

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Madriaga, M. A., L. C. McPhee, T. Chersa, K. J. Christie, and P. J. Whelan. Modulation of locomotor activity by multiple 5-HT and dopaminergic receptor subtypes in the neonatal mouse spinal cord. *J Neurophysiol* 92: 1566–1576, 2004; 10.1152/jn.01181.2003. Recently, it has been shown that bath-applied 5-HT can elicit fictive locomotion from perinatal mouse preparations. Since 5-HT acts on multiple receptor subtypes, the focus of this study was to examine which receptor families contribute to the genesis and modulation of locomotor activity. Blockade of 5-HT_2 (ketanserin or *N*-desmethylclozapine) or 5-HT_7 receptors (SB-269970) could reversibly block or modulate the locomotor-like pattern. A 5-HT_2 agonist (α-methyl-5-HT) was shown to be capable of activating the rhythm. Bath application of 5-HT_7 agonists (5-CT) generally led to a tonic increase in neurogram discharge, accompanied by bouts of rhythmic activity. Blockade of dopaminergic receptors {D_1 [*R*-(+)-SCH-23390 or LE 300]/D_2 [(±)-sulpiride or L-741,626] } could reversibly disrupt the rhythm and most effectively did so when the D_1 and D_2 antagonists were added together. Conversely, 5-HT_2 and D_1/D_2 agonists can interact to evoke locomotor activity. Overall, our data show that, in the neonatal mouse preparation, 5-HT evoked locomotion is partly dependent on activation of 5-HT_2, 5-HT_7, and dopaminergic receptor subtypes.

INTRODUCTION

Locomotor centers in the lumbar spinal cord receive serotonergic innervation from descending pathways that originate in the Raphe nucleus and terminate in the intermediate gray and the ventral horn (Ballion et al. 2002; Carlsson et al. 1963, 1964). Several studies suggest that these descending serotonergic projections may elicit and modulate locomotor behavior (Brustein et al. 2003; Cina and Hochman 2000; Cowley and Schmidt 1994; Kiehn and Kjærulff 1996; McDearmid et al. 1997; Nishimaru et al. 2000; Norreel et al. 2003; Pflieger et al. 2002; Wikstrom et al. 1995; for review, see Schmidt and Jordan 2000).

Endogenous 5-HT contributes to the development of spinal locomotor circuits (Branchereau et al. 2002; Cazalets et al. 2000; Pflieger et al. 2002). Besides these long-term trophic effects, 5-HT acts as a neuromodulator, activating interneurons comprising the spinal central pattern generator (CPG) and producing stable locomotor-like rhythms in neonatal rat (Beato and Nistri 1998; Cazalets et al. 1992; Cowley and Schmidt 1994; Kiehn and Kjærulff 1996; Schmidt and Jordan 2000; Sqalli-Houssaini et al. 1993) and mouse (Branchereau et al. 2000; Nishimaru et al. 2000; Whelan et al. 2000).

Identification of the receptors activated by 5-HT is an important step in understanding how 5-HT can produce sustained activation of CPGs. Although no work addressing this issue has been published using the mouse, several investigations have been completed using the neonatal rat spinal cord preparation. Although early studies (Cazalets et al. 1992) suggested that activation of multiple 5-HT receptors contributed during locomotion, this issue has been revisited with the availability of more specific 5-HT agonists and antagonists. Application of relatively selective 5-HT_1 agonists or 5-HT_2 antagonists inhibit ongoing evoked locomotor activity (Beato and Nistri 1998; Bracci et al. 1998; MacLean et al. 1998). This is consistent with 5-HT_2 receptors depolarizing spinal neurons (Schmidt and Jordan 2000). Preliminary evidence suggests that activation of 5-HT_7, and to a lesser extent 5-HT_2, receptors can elicit and modulate locomotor-like patterns in neonatal rats (Cina and Hochman 1998; Fyda and Jordan 1999; Schmidt and Jordan 2000). Consistent with the in vitro data, DOI (a 5-HT_2 agonist), can promote activation of lumbar locomotor patterns in vivo in spinalized neonatal rats (Norreel et al. 2003).

While it would be convenient to assume that receptor subtypes activated during fictive locomotion are similar in both rats and mice, this may not be the case. In this study, we examine, for the first time, the array of receptors contributing to 5-HT–induced locomotor activity in the neonatal mouse spinal cord preparation. Our data suggest that activation of dopaminergic receptors either by endogenous dopamine or by 5-HT contributes to the effectiveness of 5-HT–induced locomotor-like activity. These data are consistent with the proposal (Jordan and Schmidt 2002; Zaporozhets et al. 2003) that release of several monoamines (noradrenaline, dopamine, 5-HT) can evoke and/or modulate coordinated locomotor-like activity in the neonatal mouse (Jiang et al. 1999; Whelan et al. 2000) or rat (Atsuta et al. 1991; Barriere et al. 2004; Kiehn and Kjærulff 1996; Kiehn et al. 1999; Smith et al. 1988; Sqalli-Houssaini and Cazalets 2000). A portion of these results has been published in abstract form (Madriaga et al. 2002).

METHODS

Experiments were performed on Swiss Webster mice (Charles River Laboratories) that were 2–3 days old (P2–P3; $n = 73$). The animals were anesthetized by hypothermia or methoxyflurane, decapitated, and eviscerated. All procedures were approved by the University of Calgary Animal Care Committee. The remaining tissue was placed in a dissection chamber filled with oxygenated (95% O_2-5% CO_2) low calcium and high magnesium artificial cerebrospinal fluid

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(ACSF) to minimize spinal shock (in mM): 128 NaCl, 4 KCl, 0.1 $CaCl_2$, 2 $MgSO_4$, 0.5 NaH_2PO_4, 21 $NaHCO_3$, and 30 D-glucose. A ventral laminectomy was performed, and the ventral and dorsal roots were cut. The spinal cord was transected at T_5 and S_3 and gently removed from the vertebral column. The isolated spinal cord was transferred to the recording chamber and superfused with oxygenated (95% O_2-5% CO_2) ACSF (in mM): 128 NaCl, 4 KCl, 1.5 $CaCl_2$, 1 $MgSO_4$, 0.5 NaH_2PO_4, 21 $NaHCO_3$, and 30 D-glucose. The bath solution was heated gradually to 27°C from room temperature. The preparation was allowed to equilibrate for ~1 h.

Electrophysiological recordings and activation of locomotor networks

Motoneuron activity was recorded with suction electrodes into which the segmental L_2 and L_5/L_6 ventral roots were drawn. The resultant neurograms were amplified (100–20,000 times), filtered (100 Hz–1 kHz or DC–1 kHz), and digitized (Axon Digidata 1320) for future analysis. Alternating segmental and ipsilateral ventral root bursting patterns were taken to be indicative of fictive locomotion (Nishimaru et al. 2000; Whelan et al. 2000).

Either 5-HT (20–40 μM; Sigma-Aldrich) or a 5-HT_2 agonist [α-methyl-5-HT (α-m-5-HT), 2.5–4 μM; Sigma-Aldrich] was added to the bath, which caused an increase in ventral root neurogram discharge, followed by the emergence of a coordinated locomotor-like rhythm. The rhythm was allowed to stabilize over 30 min, after which antagonist or agonists were added to the bath. A washout was performed using the control 5-HT/5-HT_2 agonist concentration. In some experiments, we attempted to elicit fictive locomotion using specific agonists for the following receptors: D_1 [*R*-(+)-SKF-38393, 10–20 μM; Sigma-Aldrich], D_2 [(−)-quinpirole, 20–40 μM; Sigma-Aldrich], and 5-HT_7 (5-CT, 10–15 μM; Sigma-Aldrich, with GR-127935 and WAY-100635 to block 5-HT_1 receptors, 0.1 μM each; Sigma-Aldrich). The listed antagonists were used to block the following receptors during 5-HT–evoked fictive locomotion: D_1 [R-(+)-SCH-23390, 2–4 μM or LE 300, 0.3–1 μM; Sigma-Aldrich], D_2 [(±)-sulpiride, 10–20 μM, or L-741,626, 0.3–6 μM; Sigma-Aldrich], 5-HT_2 (ketanserin, 0.01–1 μM; Sigma-Aldrich), 5-HT_{2C} (*N*-desmethylclozapine, 0.1–1.0 μM; Sigma-Aldrich), and 5-HT_7 [SB-269970 (Lovell et al. 2000), 0.3–10 μM; Sigma-Aldrich]. Control experiments were performed to ensure that vehicles used to dissolve the drugs had no effect on the rhythm.

Data analyses

Five-minute segments of data were analyzed immediately before drug addition, 30 min after, and following washout. Data were rectified, low-pass filtered, and resampled at 100 Hz (MatLab Software, Mathworks). The mean offset was subtracted, and the data were further smoothed using a Savitzky-Golay digital filter. Cross-correlograms were calculated to measure the coupling between the left and right L_2 ventral bursts and the left or right L_2 and L_5 ventral bursts during the different experimental conditions. Stable alternating rhythms are characterized by a high negative correlation coefficients at zero phase lag followed by multiple peaks with little decrement. As the rhythm becomes interspersed with bouts of tonic uncoordinated activity, this value at zero phase lag becomes less negative and the subsequent peaks rapidly attenuate. To measure the stability of the rhythm, the peak-to-trough correlation coefficient (PTCC) was calculated from the cross-correlogram by subtracting the minimum negative value of the correlation coefficient from the maximum of the first positive peak (see Fig. 1*B*) over the first 75 lags (each lag = 50 ms). Therefore a perfectly stable alternating rhythm with a negative trough at zero would have a value of −2. When antagonists were added we often observed bouts of tonic bursting similar to spontaneous activity. The correlation difference over an interval of 75 lags will produce a small positive value, reflecting the lack of a regular rhythm. Cycle

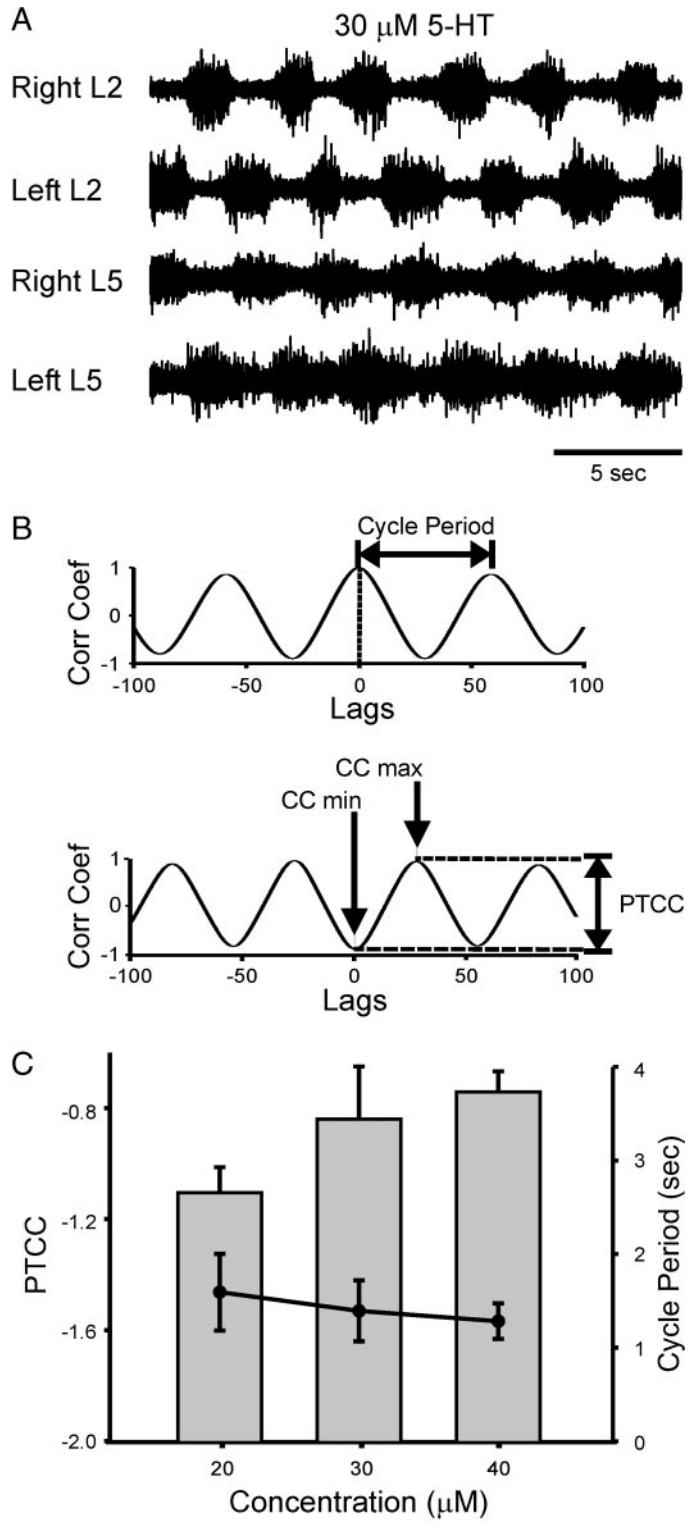

FIG. 1. Genesis of a locomotor-like rhythm following bath application of 30 μM 5-HT. *A*: neurograms show rhythmic bursting activity recorded from the L_2 and L_5 ventral roots. *B*: autocorrelogram (*top*) from a 5-min section of data; cycle periods were calculated from measuring the lags from lag 0 to the next peak (each lag = 50 ms). Stability (*bottom*) of the alternating rhythm was calculated by subtracting the maximum from the minimum value as shown [peak-to-trough correlation coefficient (PTCC); see METHODS]. *C*: dose-response graph showing stability (●) of the rhythm between left and right L_2 ventral roots at different concentrations of bath-applied 5-HT. Gray bars, cycle period; error bars, SE.

periods were calculated by measuring the number of lags from lag 0 to the first peak from the autocorrelograms. The normalized phase lag between the L_2 segmental roots was calculated from the cross-correlogram by measuring the number of lags from lag 0 to the first positive peak and dividing by the cycle period. Statistical comparisons between experimental conditions were made using a *t*-test or one-way ANOVA if the data were normally distributed and had equal variance. Otherwise, the data were compared with a Mann-Whitney rank sum test.

RESULTS

Figure 1 shows the bursting pattern recorded from the L_2 and L_5 ventral root neurograms following bath application of 5-HT. A dose-response relationship was established by bath-applying 10, 20, 30, and 40 μM 5-HT and measuring the cycle period and stability of the alternating rhythm. A stable rhythm was generally established within 5–20 min following the addition of 5-HT as has been previously reported (Nishimaru et al. 2000). Although nonsignificant, there was a trend for the rhythm to slow down in a dose-dependent fashion and for the stability of the rhythm to increase. Concentrations $<$20 μM were not successful in evoking sustained rhythmic activity. Compiling results from this study, we found that stable rhythms could be recorded from 49 preparations following bath-application of 5-HT [30 μM—L_2–L_2: -1.53 ± 0.04 (PTCC), 3.63 ± 0.14 s (cycle period), 0.51 ± 0.04 (phase lag)]. In 6/49 preparations, while an alternating L_2 rhythm was clearly evident, the activity from L_5 neurograms was either tonic or comprised of short bouts of rhythmic ipsilateral alternating activity (L_2–L_5). This is reflected in the slightly lower PTCC scores for the ipsilateral [L_2–L_5: -1.36 ± 0.08 (PTCC); 0.46 ± 0.01 (phase lag)] compared with the segmental L_2 rhythm.

Contribution of serotonergic receptor subtypes to 5-HT–evoked locomotion

We examined the contribution of 5-HT_2, 5-HT_7, D_1 and D_2 receptor subtypes to the generation of locomotor activity evoked by bath application of 30 μM 5-HT.

5-HT_2 receptors

The sustained 5-HT evoked rhythm began to break down ~10 min after bath-application of ketanserin (10–50 nM), and we noticed that rhythmic activity ceased to be recorded from L_5 neurograms before L_2 activity was affected. By ~30 min, no rhythmic activity could be recorded from the L_2 and L_5 neurograms [control—L_2–L_2: -1.42 ± 0.30 (PTCC), 2.88 ± 0.31 s (cycle period); L_2–L_5: -1.21 ± 0.18 (PTCC); ketanserin—L_2–L_2: 0.07 ± 0.19 (PTCC); L_2–L_5: 0.34 ± 0.06 (PTCC); $n = 3$; $P < 0.05$; Fig. 2]. The rhythm recovered following washout with 5-HT.

In separate experiments, we examined the effects of blocking the 5-HT_{2C} receptor. Addition of 0.25–0.50 μM *N*-desmethylclozapine blocked rhythmic activity in all preparations [control—L_2–L_2: -1.59 ± 0.03 (PTCC), 3.89 ± 0.59 s (cycle period); L_2–L_5: -0.81 ± 0.56 (PTCC); *N*-desmethylclozapine—L_2–L_2: -0.34 ± 0.33 (PTCC); $n = 3$; $P < 0.05$; L_2–L_5: 0.16 ± 0.05 (PTCC); $n = 3$; $P > 0.1$; data not shown]. Washout with ACSF and reapplication of 30–50 μM 5-HT restored the rhythm ($P > 0.1$).

5-HT_7 receptors

A specific antagonist (SB-269970) was used to block 5-HT_7 receptors (Lovell et al. 2000). Following the establishment of a regular rhythm [L_2–L_2: -1.63 ± 0.11 (PTCC), 4.31 ± 0.28 s (cycle period), $n = 4$; L_2–L_5 PTCC: -1.37 ± 0.12; $n = 3$; Fig. 3], increasing concentrations of SB-269970 (0.3–4 μM) were added to the bath at intervals of 30 min. At doses $>$0.6 μM, there was a significant reduction in the quality of the rhythm, and at 1 μM, we observed a slowing of the rhythm from 4.28 ± 0.58 to 5.42 ± 0.20 s in three of four preparations, with a suppression of the rhythm in the remaining preparation. Complete suppression of the L_2 rhythm occurred at 4 μM in three of four preparations [1 μM SB-269970: -0.87 ± 0.20 (PTCC); 4 μM SB-269970: -0.01 ± 0.07 (PTCC); $P < 0.05$; Fig. 3]. In the remaining preparation, when we increased the concentration of SB-269970 to 10 μM, the rhythm was blocked. In the three preparations that produced an L_2–L_5 rhythm, a full blockade of this rhythm occurred at doses of 4 μM SB-269970 [0.57 ± 0.06 (PTCC), $P > 0.1$]. Washout of the SB-269970 led to a resumption of the alternating bursting pattern (PTCC: $P > 0.1$ compared with control data).

Contribution of dopaminergic receptors to 5-HT–evoked locomotion

In the first series of experiments, both D_1 and D_2 receptors were blocked by 2 μM *R*-(+)-SCH 23390 and 10 μM (±)-sulpiride, respectively (Fig. 4*A*). In four of six preparations, rhythmic activity was completely blocked [control—L_2–L_2: -1.58 ± 0.12 (PTCC), 4.06 ± 0.09 s (cycle period); L_2–L_5: -1.33 ± 0.23 (PTCC); D_1/D_2 antagonists—L_2–L_2: -0.44 ± 0.26 (PTCC); $n = 6$; $P < 0.05$; L_2–L_5: 0.19 ± 0.20 (PTCC), $n = 4$, $P < 0.05$]. In the remaining two preparations, the L_2 cycle period decreased to 2.60 ± 0.94 s.

To assess which dopamine receptor family was responsible, *R*-(+)-SCH 23390 and (±)-sulpiride were added sequentially in a separate series of experiments. Addition of *R*-(+)-SCH 23390 blocked rhythmic activity in four of seven preparations [control—L_2–L_2: -1.63 ± 0.06 (PTCC), L_2–L_5: -1.47 ± 0.10 (PTCC); 4 μM SCH 23390—L_2–L_2: -0.50 ± 0.17 (PTCC); L_2–L_5: 0.40 ± 0.05 (PTCC); $n = 7$; $P < 0.05$]. In the remaining three preparations, the L_2 cycle period decreased from 3.54 ± 0.19 (control) to 1.78 ± 0.86 s (4 μM SCH 23390). Addition of 10 μM (±)-sulpiride blocked the rhythm in these three preparations (PTCC: $P < 0.05$). Washout of the antagonists and reapplication of 5-HT (30 μM) elicited a rhythm with a similar PTCC as controls ($P > 0.1$). A more specific D_1 antagonist, LE 300, was also tested with similar results [1 μM LE 300—L_2–L_2: 0.30 ± 0.09 (PTCC); L_2–L_5: 0.36 ± 0.08 (PTCC), $n = 4$, $P < 0.05$]. Rhythmic activity returned following washout of LE 300 ($P > 0.1$). Although a trend for a dose-dependent decrease in the cycle period was observed using LE 300, this was not significant.

In a separate series of experiments, (±)-sulpiride was added to the bath followed by *R*-(+)-SCH-23390 30 min later (Fig. 4*C*). In these experiments, we observed a slight reduction in the stability of the rhythm in three preparations, characterized by

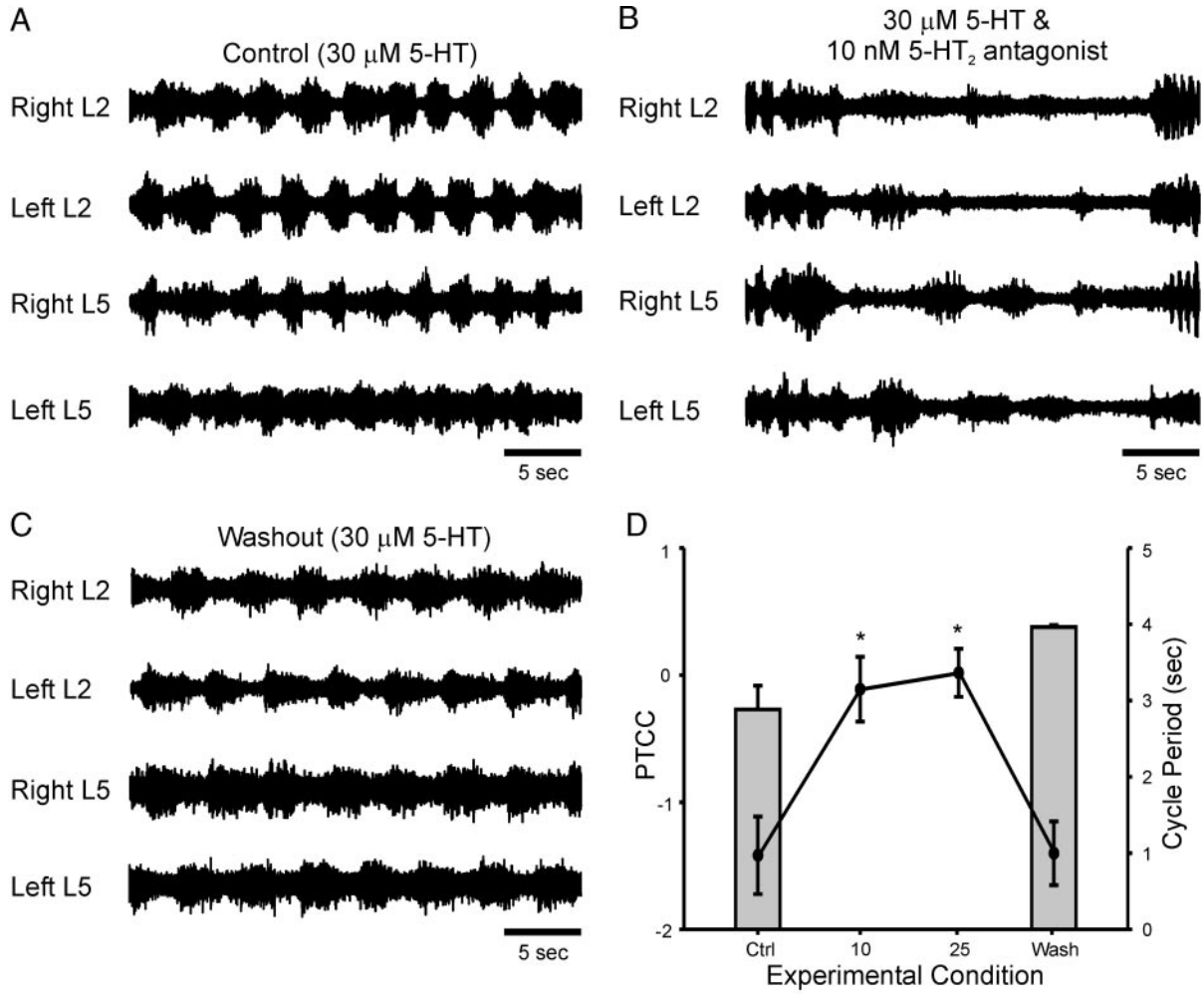

FIG. 2. Bath application of a 5-HT_2 receptor antagonist disrupts a 5-HT–evoked rhythm. *A*: 5-HT–evoked control rhythm (30 μM). *B*: 5-HT_2 antagonist (ketanserin, 10 nM) was bath-applied. *C*: washout with 30 μM 5-HT. *D*: mean L_2-L_2 PTCC (●) significantly decreased in a dose-dependent manner following addition of ketanserin (ANOVA, $P < 0.05$, $n = 3$). Corresponding cycle periods are represented by gray bars. Error bars, SE. *Significant difference.

long rhythmic bouts of activity punctuated by short tonic episodes. A complete blockade of the rhythm occurred in the remaining three preparations [control—L_2–L_2: -1.65 ± 0.12 (PTCC), 3.13 ± 0.27 s (cycle period), L_2–L_5: -1.63 ± 0.06 (PTCC); 20 μM ($\pm$)-sulpiride: L_2–L_2: -0.58 ± 0.29 (PTCC); L_2–L_5: -0.34 ± 0.22 (PTCC), $n = 6$, $P < 0.05$]. In the three preparations showing mild disruption of the rhythm, we did not observe any significant change in cycle period following the addition of ($\pm$)-sulpiride. Addition of *R*-(+)-SCH-23390 completely blocked the rhythm in these three preparations (PTCC, $P < 0.05$). The effects of a new, more specific D_2 antagonist, L-741,626 (0.3–10 μM), were also tested. At lower doses (0.3–0.9 μM), no significant disruption of the rhythm ensued ($n = 4$, $P > 0.1$). However, in a separate series of experiments, at higher doses (2–10 μM), the rhythm was blocked in four of five preparations (PTCC: $P < 0.05$).

Contribution of 5-HT and dopaminergic receptor subtypes to locomotor-like activity

In the next set of experiments we examined the ability of 5-HT and dopaminergic receptor subtype agonists to generate locomotor-like activity.

5-HT_2 receptor agonists and interactions with D_1/D_2 receptors

We first tested whether application of 5-HT_2 receptor agonists were sufficient to evoke alternating rhythmic activity. Following the addition of 4 μM of α-m-5-HT, rhythmic activity could be recorded from the L_2 ventral roots [L_2–L_2: -1.47 ± 0.11 (PTCC), 0.44 ± 0.01 (phase lag), $n = 9$; Fig. 5, *A* and *B*]. The cycle period calculated from the right L_2 autocorrelogram was 4.06 ± 0.43 s. However, there were some differences in the actions of α-m-5HT compared with 5-HT. The rhythm tended to take somewhat longer to become stable (about 20 min) using α-m-5HT. Also, rhythmic activity was recorded from the L_5 ventral roots from only three of nine preparations [L_2–L_5: -1.44 ± 0.03 (PTCC); 0.56 ± 0.01 (phase lag); $n = 3$]. Addition of 0.10–0.15 μM of ketanserin completely blocked the ongoing rhythm in three of three preparations [L_2–L_2: -0.15 ± 0.26 (PTCC); L_2–L_5: 0.62 ± 0.12 (PTCC); $n = 3$; $P < 0.05$], suggesting that the α-m-5-HT was acting through 5-HT_2 receptors. In one preparation, the rhythm slowed down with 0.10 nM ketanserin (shown in Fig. 5*C*) and was blocked following addition of 0.15 nM.

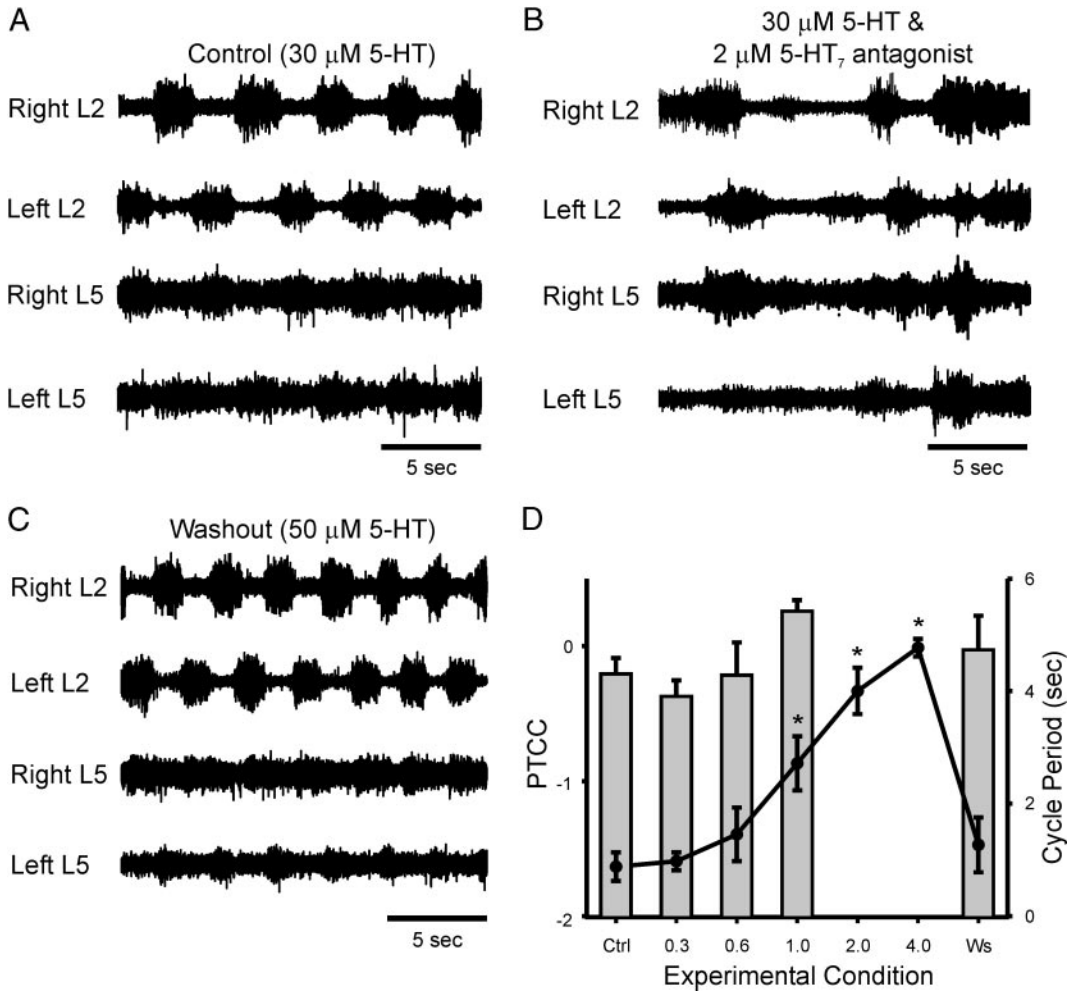

FIG. 3. Rhythm is disrupted following addition of a 5-HT_7 antagonist. *A*: control rhythm evoked by bath application of 5-HT (30 μM). *B*: addition of 5-HT_7 antagonist (SB-269970, 2 μM) to the bath. *C*: washout with 5-HT (50 μM). *D*: dose-dependent decrease in mean stability of L_2-L_2 rhythm (●) following addition of SB-269970. *Significant difference (ANOVA, $P < 0.05$). Gray bars, cycle period; error bars, SE.

Given the rhythmogenic effects of 5-HT_2 agonists and dopaminergic receptor involvement in 5-HT–evoked locomotion, we tested the hypothesis that D_1 and D_2 receptor agonists could evoke a rhythm when combined with low concentrations of 5-HT_2 receptor agonists. A concentration of α-m-5-HT (<2.5 μM) was bath-applied, which resulted in the production of tonic or uncoordinated burst discharge from the ventral roots [L_2–L_2: −0.43 ± 0.04 (PTCC), L_2–L_5: −0.44 ± 0.16 (PTCC), $n = 3$, Fig. 6*A*]. Following the bath application of D_1 and D_2 agonists [15 μM *R*-(+)-SKF-38393 and 35 μM (−)-quinpirole, respectively), a significantly more stable rhythmic pattern from segmental and ipsilateral ventral root neurograms was produced [L_2–L_2: −1.64 ± 0.08 (PTCC), 3.82 ± 0.29 s (cycle period), 0.49 ± 0.01 (phase lag); L_2–L_5: −1.47 ± 0.13 (PTCC), 0.47 ± 0.02 (phase lag); $n = 3$; $P < 0.05$; Fig. 6*B*].

Addition of only *R*-(+)-SKF-38393 to a bath containing 2.5 μM α-m-5-HT produced bouts of rhythmic activity interspersed with bouts of uncoordinated or tonic activity reflected in the lower PTCC scores ($n = 4$, $P > 0.1$, Fig. 6*C*). Following the addition of the second agonist, (−)-quinpirole, to the bath, the stability did not change significantly ($n = 4$, $P > 0.1$, Fig. 6*C*). In separate experiments, addition of the D_2 agonist, (−)-quinpirole, along with α-m-5-HT, produced sporadic bouts of rhythmic activity. Subsequent addition of *R*-(+)-SKF-38393 did produce bouts of coordinated rhythmic activity; however, the stability of the rhythm was not significantly greater than controls (PTCC: $P > 0.1$).

We examined whether bath application of dopamine or D_1/D_2 receptor agonists could elicit a rhythm, as had been reported using neonatal rats (Barriere et al. 2004; Kiehn and Kjærulff 1996). Although an increase in tonic and sporadic bursting occurred, we were not successful in eliciting a rhythm using any combination of D_1 or D_2 [≤40 μM, (−)-quinpirole] receptor agonists. Although high concentrations of dopamine (200 μM) produced bouts of locomotor-like activity, this was interspersed with long sections of tonic activity, leading to relatively low PTCC scores [L_2–L_2: −0.26 ± 0.44 (PTCC), 3.74 ± 0.08 s (cycle period); L_2–L_5: −0.14 ± 0.29 (PTCC), $n = 4$]; lower concentrations (50–100 μM) elicited bouts of uncoordinated activity (data not shown).

5-HT_7 receptor agonists

We examined whether 5-HT_7 agonists could evoke rhythmic activity (Fig. 7). First, 5-HT_{1A} and 5-HT_{1D} receptors were blocked with 0.1 μM each of WAY-100635 and GR-127935,

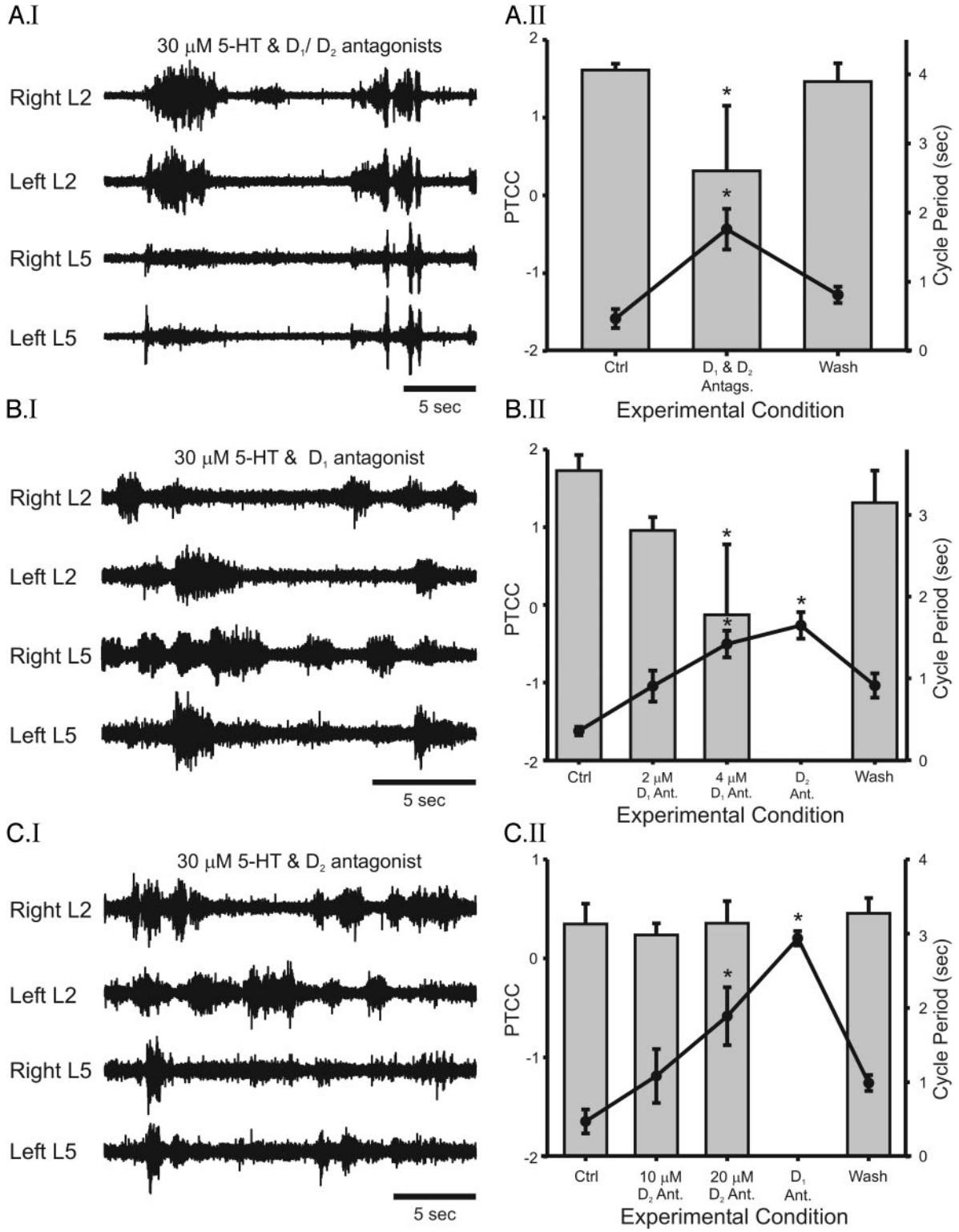

FIG. 4. Application of dopaminergic antagonists disrupts the alternating rhythmic discharge. *A.I*: addition of *R*-(+)-SCH 23390 (2 μM) and (±)-sulpiride (10 μM) caused a suppression of rhythmic activity. *A.II*: mean decrease in L_2-L_2 stability (●) and cycle period (gray bars) (ANOVA, $P < 0.05$, $n = 6$). *B.I*: serial addition of *R*-(+)-SCH 23390 caused a dose-dependent decrease in the stability and cycle period of the rhythm. *B.II*: similar to *A.II* ($n = 7$). *C.I*: addition of (±)-sulpiride decreased the stability of the rhythm. *C.II*: similar to *A.II* ($n = 6$). Error bars, SE. *Significant difference.

respectively. 5-CT (10 μM), a 5-$HT_{1/7}$ receptor agonist, was concurrently added. In six of seven preparations, an increase in tonic bursting in all ventral roots occurred. In two of seven preparations, bouts of rhythmic activity were recorded [L_2–L_2: -0.74 ± 0.06 (PTCC), L_2–L_5: -0.23 ± 0.40 (PTCC)]. The associated cycle period was 2.86 ± 0.04 s, calculated from the

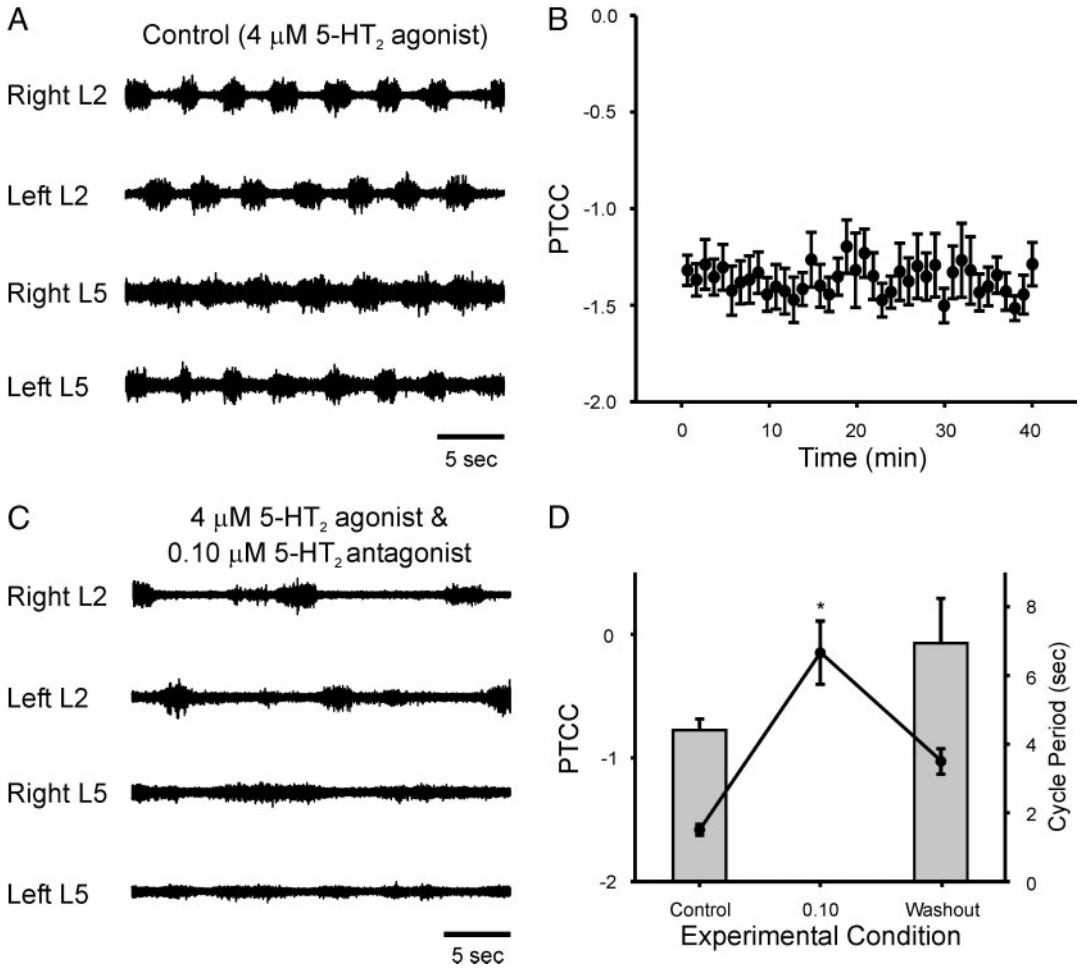

FIG. 5. Alternating rhythmic discharge can be evoked by addition of α-m-5-HT. *A*: control rhythm evoked by addition of 4 μM α-m-5-HT. *B*: long-term stability of α-m-5-HT–evoked rhythm. Stability of coupled L_2 rhythm plotted as PTCC values every minute for 40 min following onset of the rhythm ($n = 6$). *C*: rhythm was modulated by addition of 0.10 μM ketanserin (same preparation as in *A*; note rhythm was completely blocked following addition of 0.15 μM ketanserin in this preparation). *D*: change in segmental L_2 stability of the rhythm following administration of ketanserin (●, $n = 3$). *Significant difference (ANOVA, $P < 0.05$). Gray bars, cycle period; error bars, SE.

left L_2 autocorrelogram, and the phase lag was 0.48 ± 0.02 between the segmental L_2 neurograms and 0.62 ± 0.03 between the ipsilateral L_2–L_5 roots.

DISCUSSION

In the rat and mouse, it has been reported that bath application of 5-HT alone can activate spinal locomotor circuits. This work shows that locomotor-like activity produced by bath-applied 5-HT in the isolated mouse spinal cord preparation depends on activation of multiple serotonergic and dopaminergic receptor subtypes (see Table 1).

Contribution of 5-HT family of receptors to fictive locomotor activity

5-HT activates seven distinct receptor families that allow a multiplicity of actions through different signaling pathways. In the neonatal rat, it is generally thought that 5-HT_1 receptors are inhibitory to locomotor rhythmogenesis (Beato and Nistri 1998; Schmidt and Jordan 2000). In the same preparation, 5-HT_2 appears to have an excitatory effect on rhythmogenesis (Bracci et al. 1998; Cazalets et al. 1992; MacLean et al. 1998), and our data confirm that this is also the case in the neonatal mouse. However, the reliance on multiple receptor subtypes to generate sustained rhythms across rostral and caudal lumbar segments was evident in our study. For example, while 5-HT produced locomotor-like activity in most preparations, α-m-5HT produced sustained rhythmic activity mainly in rostral lumbar segments. In light of our findings that 5-HT alone activated D_1 and D_2 receptors, it is interesting that more reliable activation of caudal lumbar segments could be observed when subthreshold concentrations of 5-HT_2 were applied along with D_1/D_2 receptor agonists. One possibility is that differential distribution of 5-HT_2 and dopamine receptors in the thoracolumbar spinal cord may contribute to the effective activation of fictive locomotion. While little data exist on this issue in the mouse, data from the rat suggest that 5-HT_7 receptors are localized in the T_{11}–L_1 segments, whereas 5-HT_2 receptors are localized mainly in lumbar segments (Hochman et al. 2001; Schmidt and Jordan 2000). D_1 receptors appear widely expressed throughout the lumbar spinal cord in adult rats (Dubois et al. 1986); however, D_2 receptors show more

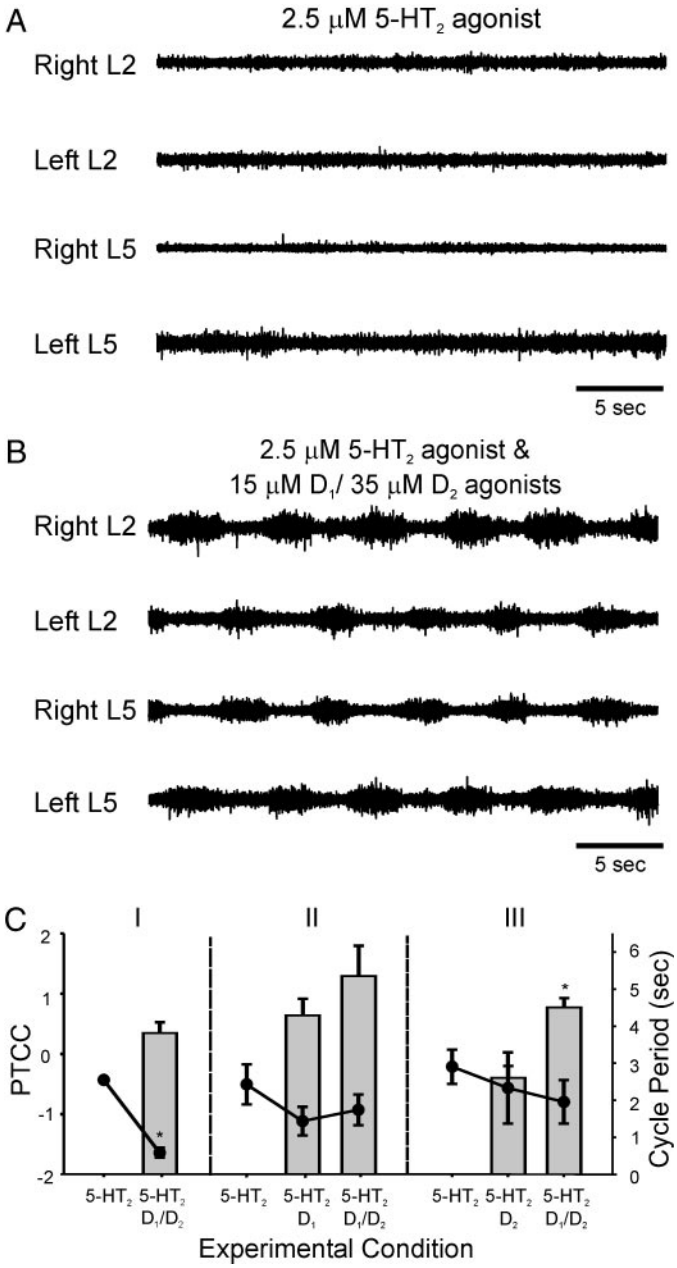

FIG. 6. Functional interaction between 5-HT_2, D_1, and D_2 receptors. *A*: addition of a low concentration of α-m-5-HT (<2.5 μM) evoked only tonic activity. *B*: concurrent addition of 15 μM *R*-(+)-SKF-38393 and 35 μM (−)-quinpirole evoked a stable alternating rhythm. *C*: mean segmental L_2 PTCC values (●) and cycle periods (gray bars). *CI*: addition of 2.5 μM α-m-5-HT followed by concurrent bath application of *R*-(+)-SKF-38393 (15 μM) and (−)-quinpirole (35 μM). *CII*: addition of α-m-5-HT (2.5 μM), followed by addition of *R*-(+)-SKF-38393 (15 μM) and then (−)-quinpirole (35 μM). *CIII*: addition of α-m-5-HT (2.5 μM), followed by (−)-quinpirole (35 μM) and then *R*-(+)-SKF-38393 (15 μM). *Significant differences ($P < 0.05$). Error bars, SE.

localized expression (Van Dijken et al. 1996). Therefore the opportunity may exist for differential modulation of different spinal segments by monoamines. This may account for the increased effectiveness of monoaminergic combinations. Another possibility is that a developmental rostrocaudal gradient of excitability (Bonnot et al. 1998) and/or differential maturation of flexor and extensor motoneurons (Vinay et al. 2000) contribute to the differential sensitivity of spinal segments to 5-HT_2 receptor agonists. Clearly, more data are required to clarify these issues.

We found that 5-HT contributed to the modulation and stability of locomotor-like activity by binding to 5-HT_7 as well as 5-HT_2 receptors. This also supports preliminary reports on this issue in the neonatal rat preparation (Cina and Hochman 1998; Fyda and Jordan 1999; Schmidt and Jordan 2000). The signaling mechanisms of 5-HT_7 actions on spinal neurons are undetermined. However, in CA1 neurons of the hippocampus, 5-HT_7 actions are mediated through inhibition of a Ca^{2+} activated K^+ conductance, leading to a reduction in the afterpolarization of neurons. Functionally, this leads to an increase in burst discharge (Gill et al. 2002). Since 5-HT_7 and 5-HT_2 receptors appear to use separate G-protein–coupled pathways (G_s and $G_{q/11}$, respectively), it suggests that 5-HT is depolarizing ventral neurons through multiple G-protein–coupled signaling mechanisms. In addition to 5-HT_2 and 5-HT_7 receptors, other receptor subtypes such as 5-HT_4 and 5-HT_6 coupled to G_s pathways (similar to 5-HT_7) could contribute to depolarization of neurons. Activation of several G-protein–coupled pathways is consistent with the modulation of multiple ionic conductances by 5-HT in spinal motoneurons (Kjaerulff and Kiehn 2001; Rekling et al. 2000; Schmidt and Jordan 2000).

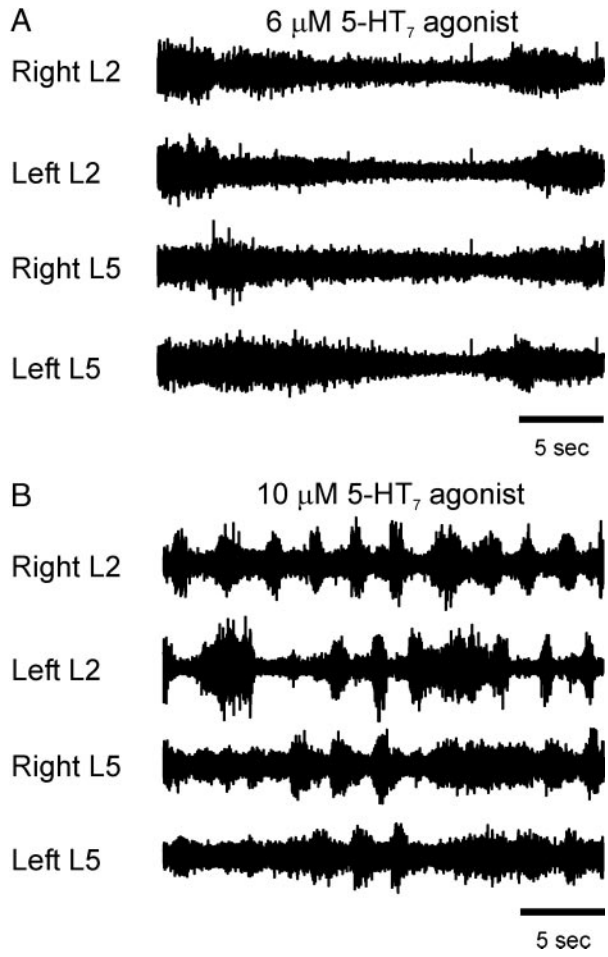

FIG. 7. Activation of 5-HT_7 receptors evoked rhythmic activity in a minority of preparations. *A*: tonic activity following addition of 6 μM 5-CT. *B*: same preparation with the dose increased to 10 μM. *A* and *B*: bath also contained 0.1 μM each of WAY-100635 and GR-127935.

TABLE 1. *Contribution of 5-HT and dopaminergic receptor subtypes to fictive locomotor activity*

Agonist/Antagonist	Receptor Family	Activity Induced	Activity Blocked or Modulated
Receptor antagonists			
Ketanserin	5-HT_2		Blocked (3/3)
N-desmethylclozapine	5-HT_{2C}		Blocked (3/3)
SB-269970	5-HT_7		Blocked (4/4)
SCH 23390 and sulpiride	D_1 and D_2		Blocked (4/6)/modulated (2/6)
SCH 23390	D_1		Blocked (4/7)/modulated (3/7)
LE 300	D_1		Blocked (4/4)
Sulpiride	D_2		Blocked (3/6)/no effect (3/6)
L-741, 626 (2–10 μM)	D_2		Blocked (4/5)/no effect (1/5)
Receptor agonists			
α-m-5-HT	5-HT_2	Yes L2-L2 (9/9); L2-L5 (3/9)	
α-m-5-HT (low conc.) + SKF-38393 + quinpirole	5-HT_2, D_1, D_2	Yes (3/3)	
α-m-5-HT (low conc.) + SKF-38393	5-HT_2, D_1	Partial (3/4)	
α-m-5-HT (low conc.) + quinpirole	5-HT_2, D_2	No (4/4)	
5-CT (5-HT_1 receptors blocked)	5-HT_7	Partial (2/7)	

Receptor antagonist effects refer to the ability of the listed compounds to modulate or block rhythmic activity induced by bath application of 5-HT. The receptor agonist effects refer to the ability of the listed compounds to induce rhythmic activity.

Contribution of dopaminergic receptors to 5-HT–evoked rhythmogenesis

Bath application of D_1/D_2 receptor antagonists led to a decrease in the stability of the 5-HT–evoked alternating rhythm. 5-HT has differing binding affinities for both the 5-HT subfamily of receptors *and* other receptors, including the dopaminergic family of receptors. Low concentrations of 5-HT ($<$10 μM) combined with either dopamine or *N*-methyl-D,L-aspartate (NMA) can produce a stable rhythm in rats and mice (Jiang et al. 1999; Sqalli-Houssaini et al. 1993; Whelan et al. 2000). At higher concentrations (10–100 μM), 5-HT alone can activate spinal locomotor circuits (Bracci et al. 1998; Cazalets et al. 1992; Kiehn and Kjærulff 1996; MacLean et al. 1998; Nishimaru et al. 2000; Sqalli-Houssaini et al. 1993). Our data suggest that 5-HT at high concentrations may be cross-reacting and activating the D_1 and D_2 receptor families. While we consider this a likely explanation, other possibilities must be considered.

First, dopamine could be endogenously released in the isolated spinal cord and may contribute to activation of the CPG. Endogenous release of monoamines in the spinal cord is a feature of simpler vertebrates such as the lamprey and has been found to affect rhythmicity (Kemnitz et al. 1995; McPherson and Kemnitz 1994; Pierre et al. 1997; Svensson et al. 2003). In the mouse spinal cord, only a few neurons immunoreactive to 5-HT are found mainly in sacral segments (Ballion et al. 2002). Nevertheless, it has been found that blocking 5-HT receptors can affect *N*-methyl-D-aspartate (NMDA)-evoked locomotion, suggesting a possible role for endogenous release of monoamines (MacLean et al. 1998). Future studies will need to tackle this possibility.

A second possibility is that SCH 23390 binds to 5-HT_{2C} receptors, where it acts as a partial agonist (Briggs et al. 1991), and could disrupt the rhythm. However, several lines of evidence mitigate this possibility. First, activation of 5-HT_{2C} receptors by SCH-23390 would be expected to increase the excitability of locomotor networks and would not explain the general reduction in rhythmicity and excitability that we observed. Second, a more selective D_1 antagonist blocked the rhythm. Finally, the rhythm was most effectively blocked when a combination of D_1 and D_2 receptor antagonists were used.

A third possibility is that activation of dopamine receptors are simply contributing to the depolarization of motoneurons and not affecting the CPG. If motoneurons alone were targeted, one would expect the timing of the rhythm to remain unaltered, although differences in the pattern of bursting behavior could occur. Our data show that dopaminergic antagonists destabilized the 5-HT evoked rhythm and increased the frequency, suggesting that interneurons comprising the CPG were affected. Nevertheless, the contribution of motoneurons to rhythm generation cannot be discounted, since in the presence of TTX, a stable rhythm can be recorded from populations of neonatal rat motoneurons coupled by way of gap junctions (Tresch and Kiehn 2000).

5-HT–evoked activity also partly depended on activation of D_2 receptors. Findings from the neonatal rat also suggest that both D_1 and D_2 receptors contribute to locomotor activity induced by dopamine (Barriere et al. 2004). However, similar to our results, D_1 receptor activation was found to primarily contribute to rhythm generation. While D_1 and D_2 receptors are coupled to G_s and G_i pathways and activate and inhibit the production of adenyl cyclase, respectively (Missale et al. 1998), evidence exists for D_1/D_2 pathways working synergistically to produce behaviorally appropriate output in brain and spinal cord (Barriere et al. 2004; Braun and Chase 1986; LaHoste and Marshall 1990; LaHoste et al. 2000).

Functional considerations

It has been reported that high concentrations of bath-applied 5-HT are sufficient to generate locomotor-like activity in the neonatal mouse (Nishimaru et al. 2000). This study shows that, at high concentrations of bath-applied 5-HT, dopaminergic receptors are activated and facilitate the expression of fictive locomotor activity.

A preliminary report using the neonatal rat brain stem–spinal cord preparation suggests that locomotor activity elicited by electrical activity of the brain stem can be blocked by adding antagonists to dopaminergic, serotoninergic, or noradrenergic receptors (Zaporozhets et al. 2003). This adds credence to the argument that catecholamine and serotonergic signaling pathways interact to produce locomotor behavior, as has been suggested using in vivo neonatal rat models (McEwen et al.

1997). At birth, descending monoaminergic systems are not fully developed (Schmidt and Jordan 2000). Convergent release of multiple monoamines and other peptides may be necessary to activate thoracolumbar CPG circuits. Relatively high concentrations of dopamine, noradrenaline, and 5-HT have been found within T_{11}–T_{13} spinal segments in neonatal rats following brain stem stimulation (Jordan and Schmidt 2002). Our data show dopamine and 5-HT can interact to produce sustained locomotor-like activity. In the future, it will be important to test, at the cellular level, the signaling mechanisms that contribute to the combinatorial effects of monoamines on spinal neurons.

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