Effects of Noradrenaline on Locomotor Rhythm–Generating Networks in the Isolated Neonatal Rat Spinal Cord

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Kiehn, Ole, Keith T. Sillar, Ole Kjaerulff, and Jonathan R. McDearmid. Effects of noradrenaline on locomotor rhythm–generating networks in the isolated neonatal rat spinal cord. J. Neurophysiol. 82: 741–746, 1999. We have studied the effects of the biogenic amine noradrenaline (NA) on motor activity in the isolated neonatal rat spinal cord. The motor output was recorded with suction electrodes from the lumbar ventral roots. When applied on its own, NA (0.5–50 μM) elicited either no measurable root activity, or activity of a highly variable nature. When present, the NA-induced activity consisted of either low levels of unpatterned tonic discharges, or an often irregular, slow rhythm that displayed a high degree of synchrony between antagonistic motor pools. Finally, in a few cases, NA induced a slow locomotor-like rhythm, in which activity alternated between the left and right sides, and between rostral and caudal roots on the same side. As shown previously, stable locomotor activity could be induced by bath application of N-methyl-D-aspartate (NMDA; 4–8.5 μM) and/or serotonin (5-HT; 4–20 μM). NA modulated this activity by decreasing the cycle frequency and increasing the ventral root burst duration. These effects were dose dependent in the concentration range 1–5 μM. In contrast, at no concentration tested did NA have consistent effects on burst amplitudes or on the background activity of the ongoing rhythm. Moreover, NA did not obviously affect the left/right and rostrocaudal alternation of the NMDA/5-HT rhythm. The NMDA/5-HT locomotor rhythm sometimes displayed a time-dependent breakdown in coordination, ultimately resulting in tonic ventral root activity. However, the addition of NA to the NMDA/5-HT saline could reinstate a well-coordinated locomotor rhythm. We conclude that exogenously applied NA can elicit tonic activity or can trigger a slow, irregular and often synchronous motor pattern. When NA is applied during ongoing locomotor activity, the amine has a distinct slowing effect on the rhythm while preserving the normal coordination between flexors and extensors. The ability of NA to “rescue” rhythmic locomotor activity after its time-dependent deterioration suggests that the amine may be important in the maintenance of rhythmic motor activity.

INTRODUCTION

The neural networks of the vertebrate spinal cord that are responsible for generating rhythmic locomotor movements are capable of producing a wide spectrum of outputs in which the intensity, duration, cycle frequency, and phasing of the motor bursts are variable (Kiehn et al. 1997; Sillar et al. 1997). This flexibility in motor output is important because rhythmic movements (swimming or walking, for example) must be able to adapt to changing behavioral requirements. Some of this flexibility in motor rhythm generation is accomplished by inputs from peripheral sense organs. However, the motor output can also be changed centrally through neuromodulation of the spinal networks themselves. Within the rhythm and pattern-generating networks, neuromodulators can act on both the electrical properties of neurons and on their synaptic interconnections (Kiehn and Katz 1999). Some of the neuromodulators are intrinsic to the spinal networks themselves, whereas others originate in centers outside the spinal cord such as the brain stem (Katz 1995). Prominent among the extrinsic sources of neuromodulation are noradrenergic (NA) and serotonergic (5-HT) neurons of locus coeruleus and the raphe nuclei, respectively.

There is now a wealth of information on the actions of 5-HT on spinal motor networks. 5-HT is able to initiate (Cazalets et al. 1992; Kiehn and Kjaerulff 1996; Viala and Buser 1969), or modulate (Barbeau and Rossignol 1991; Harris-Warrick and Cohen 1985; Sillar et al. 1992) locomotor activity in all vertebrates studied so far. These actions, mediated at a range of 5-HT receptor subtypes (Wallis 1994), are accomplished via effects of 5-HT on cellular properties (Sillar and Simmers 1988), synaptic effects of 5-HT on spinal networks, this amine is also able to initiate stable locomotor activity in the cat and rabbit (Barbeau and Rossignol 1991; Chau et al. 1998; Forssberg and Grillner 1973; Jankowska et al. 1967; Kiehn et al. 1992; Viala and Buser 1969) and to slow down the ongoing locomotor rhythm in the cat (Rossignol et al. 1998) and the Xenopus tadpole (McDearmid et al. 1997). In the tadpole, a relatively simple lower vertebrate preparation, NA strengthens reciprocal inhibition via a presynaptic facilitation of glycine release from commissural interneurons (McDearmid et al. 1997). It has been proposed that this mechanism is largely responsible for the slowing effect of NA on swimming. However, whether this modulatory action on glycinergic synapses is phylogenetically conserved in higher vertebrates, or whether other cellular mechanisms may be involved in the slowing effect of NA on locomotor activity is still unknown. The purpose of the present study, therefore, was to initiate a study on the cellular, synaptic, and network actions of NA in the spinal cord of higher vertebrates. For this purpose we have used a relatively simple mammalian preparation, the isolated spinal cord of the neonatal rat. The effects of NA (unlike 5-HT) on locomotor activity in the neonatal rat have yet to be addressed in any detail (Cazalets et al. 1990). We therefore have begun to examine the modulatory role of NA on rhythm and pattern generation in the neonatal rat, by investigating the transmitter’s ability to induce
rhythmic motor activity and to modulate various parameters of the ongoing locomotor pattern.

METHODS

Dissection

Preparations of the spinal cord \( (n = 14) \) were isolated from neonatal rats 0–2 days old. Details of the dissecting procedure have been described previously (Kiehn and Kjaerulff 1996), but, briefly, the rats were first deeply anaesthetized with ether, then decapitated and eviscerated. The spinal cord extending from C1 to L6, including ventral and dorsal roots, was then isolated and the preparation transferred to a recording chamber and superfused with oxygenated \((95\% O_2 - 5\% CO_2)\) Ringer solution of the following composition (in mM): 128 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.25 MgSO4, 25 NaHCO3, 2.5 CaCl2, and 20 glucose, pH 7.4. Experiments were performed at room temperature.

Induction of rhythmic locomotor activity

Rhythmic locomotor activity was induced in the spinal cord by the bath application of N-methyl-D-aspartic acid (NMDA, 4–8.5 μM) alone or in combination with 5-hydroxytryptamine (5-HT, 4–20 μM). With the composition of the extracellular medium used in our laboratory, these drug concentrations usually give stable long-lasting locomotor-like activity (see below and Iizuka et al. 1997; Kiehn and Kjaerulff 1996; Kjaerulff and Kiehn 1996). The preparation would be considered healthy as long as 5-HT/NMDA could produce a locomotor-like motor output. Norepinephrine (NA, 0.5–50 μM) was added either on its own (Fig. 2), together with NMDA/5-HT from the start of the experiment (Fig. 3), or during ongoing activity induced by administration of 5-HT and/or NMDA (Fig. 5). All drugs were obtained from Sigma (St. Louis, MO).

Recordings

Activity in the L2 and L5 ventral roots on both sides of the cord was recorded with suction electrodes. Activity in these roots corresponds to flexor and extensor activity, respectively (Kiehn and Kjaerulff 1996). Root recordings were band-pass filtered (100–10,000 Hz), digitized, stored on a digital tape recorder (Biologic DTR 1800; Claix, France), and printed on thermosensitive paper (Gould 4000; Valley View, OH).

Analysis

Ventral root recordings were digitized (1,000 Hz) off-line, sampled by the Axoscope software program (version 7.0, Axon Instruments), and analyzed using a custom-designed program written in the Matlab (The MathWorks) environment. To characterize the rhythm quantitatively we measured several parameters of the ventral root activity under each experimental condition. The ventral root signal (usually in L2, in which the amplitude modulation is often most clear) was rectified and low-pass filtered. The minimal voltage (Vval, for valley) and the corresponding time (Tval) were determined in each locomotor cycle (Fig. 1C). The locomotor cycle duration was defined as the time between the minimal voltage in consecutive cycles (Tval \( n+1 \) – Tval \( n \), Fig. 1C). The peak voltage (Vpk), and the time, Thalf \( \nu \), taken to reach the voltage halfway between Vval and Vpk on the up-slope of the burst, and the corresponding Thalf \( \delta \), on the down-slope of the burst were also used. Thalf \( \nu \) – Thalf \( \delta \) (the half-width) was used to measure the ventral root burst duration. Vval and Vpk – Vval defined the interburst amplitude and the burst amplitude modulation, respectively. Mean values in individual experimental conditions were obtained by averaging between 10 and 40 consecutive cycles. All parameters were normalized by dividing with control values, and statistical significance between control and NA applications were tested with Student’s t-test. Differences in the NA effects for different ranges of drug concentrations were tested by ANOVA. The level of statistical significance was set at 0.05. Values in the text are means ± SD.

RESULTS

NA-induced motor activity

NA was applied in 31 experiments at concentrations in the range of 0.5–50 μM (8.9 ± 12.0 μM, mean ± SD). NA elicited no ventral root response \((n = 8); 3.6 ± 6.7 μM; range 0.5–20 μM; Fig. 2A), tonic ventral root activity \((n = 5); 5.4 ± 8.2 μM; range 1–20 μM), or a rhythmic motor output \((n = 18); 11.6 ± 13.7; range 1–50 μM; Fig. 2, B–C). There was no significant ascending concentration dependency for these responses in the population of animals examined. However, such a dependency was found in individual animals (Fig. 2), where low concentrations of NA elicited no ventral root activity, higher concentrations evoked tonic discharge, and the highest concentrations led to rhythmic activity. There was no obvious relationship between the cycle period and the transmitter con-
centration. The rhythmic motor responses were characterized by relatively long cycle periods of 42.4 ± 24.8 s (range 4–76 s, determined 8–10 min after onset of transmitter superfusion). In two experiments the NA-induced motor rhythms involved regular left-right (l-L2/r-L2) and rostrocaudal (l-L2/l-L5 and r-L2/r-L5) alternation, similar to (but slower than; cycle periods 4–14 s) the rhythmic activity observed with 5-HT/NMDA (e.g., Fig. 1B) or 5-HT and NMDA alone (Cazalets et al. 1992; Cowley and Schmidt 1994; Kjaerulff and Kiehn 1996). In the remaining experiments the rhythm was often irregular with a large propensity for synchronous bursts in roots that usually alternate, but with alternation intermingled. An example of this is shown in Fig. 2C where the large bursts in l-L2/r-L2 are synchronous, as are the lower amplitude bursts in all four roots. However, the large bursts are also alternating with the smaller bursts. These experiments with NA alone show that this transmitter is able to trigger a rhythmic motor output with a coordination that in most cases does not resemble any of the previously described transmitter-induced rhythms in the neonatal rat spinal cord (see Kiehn et al. 1997).

Noradrenergic modulation of an ongoing rhythm

NMDA and 5-HT were first applied to induce long-lasting rhythmic activity so that modulatory effects of bath-applied NA on locomotor activity could be investigated. In the experiments included in this study the rhythmic activity was always alternating between L2 ventral roots on either side of the cord and between L2 and L5 on the same side of the cord. We will refer to this coordination pattern as locomotor activity (Cowley and Schmidt 1994; Iizuka et al. 1997; Kiehn and Kjaerulff 1996). As has been reported previously, the 5-HT/NMDA–induced locomotor activity recorded in the neonatal rat shows time-dependent changes in frequency (Sqalli-Houssaini et al. 1993). Usually the cycle periods are long initially and then progressively shorten until they reach a plateau; 4–6 min after the initial bursts are observed. The sequences of locomotor activity used for analysis in the present study were taken during this plateau phase at comparable times after the onset of the transmitter perfusion (5-HT/NMDA alone or in combination with NA). There was always a period of at least 15 min wash in control saline between drug applications. During the wash out period the ventral root activity would vanish and eventually disappear usually 5–10 min after initiating the wash. In some experiments NA was added during the plateau phase.

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of a 5-HT/NMDA– or NMDA-induced rhythm. Sequences of locomotor activity taken from this period were then compared with equivalent locomotor sequences when a new plateau phase was reached. Under these conditions NA significantly (Student’s t-test) increased cycle periods compared with control values (n = 22, Figs. 3 and 4). The strength of this slowing effect increased in the range 1–5 μM (control cycle periods normalized to 100%; 1 μM, n = 7, cycle period = 136 ± 34%; 2–5 μM, n = 6, 169 ± 52; Figs. 3, A–C, and 4A; the population data for 1 μM and 2–5 μM were not significantly different, ANOVA) but with an apparent ceiling at higher concentrations (>5 μM, n = 9, 164 ± 47, Fig. 4A). The slowing effect could be detected at NA concentrations, which produced no motor responses when applied alone (Fig. 3E). The effect was reversible (wash, n = 10, 95 ± 16, Figs. 3D and 4A). In general the changes in period were accompanied by a concentration-dependent increase in burst duration (1 μM, 134 ± 44; 2–5 μM, 164 ± 52; >5 μM 154 ± 54; wash, 97 ± 20, Figs. 3, A–C, and 4B). However, in the presence of NA the duty cycle (burst duration divided by period length) was comparable with that of control so that burst durations and cycle periods seem to co-vary. The other locomotor parameters, that is the burst amplitude (Vpk–Vval; Fig. 4C) and the amplitude of the interburst activity (Vval; not illustrated in Fig. 4), did not change systematically, although there was a tendency for the burst amplitude to increase in some but not all ventral roots at higher NA concentrations (see Fig. 3, A–C). Finally, the left/ right and rostrocaudal alternation of the NMDA/5-HT rhythm was not obviously affected by NA application at any concentration tested (Fig. 3, A–C). We conclude from these experiments that NA has a slowing effect on the ongoing rhythm but has little effect on other locomotor parameters.

**Restoration of rhythmic locomotor activity by NA**

In the majority of experiments, the cycle periods of the NMDA/5-HT or NMDA rhythms started out with initially long periods and then shorten until they reach a plateau (see previous section). In some experiments the progressive shortening of the cycle periods of locomotor episodes induced by these drugs (Fig. 5A and B) was followed by a complete breakdown of the rhythm (Fig. 5C), which deteriorated into tonic activity (see also Sqalli-Houssaini et al. 1993). However, bath applications of NA (still in the presence of NMDA/5-HT; Fig. 5D) were able to restore a well-coordinated locomotor rhythm, similar to that occurring before the breakdown but usually at a lower average frequency. The changes in cycle duration (Fig. 5E, ◇) for this episode of events are shown in Fig. 5E along with the changes in burst duration (Fig. 5E, ▪). Corresponding decreases or increases in burst duration followed the variations in cycle duration. Figure 5F shows the changes in peak burst amplitude (Vpk, ▽), and interburst amplitude (Vval, ◆) for the same episode. Note that the peak amplitude decreases while the interburst amplitude increases before the breakdown, leading to a decrease in modulation amplitude. NA restored the modulation amplitude.

This “rescuing” of the locomotor activity was seen in five of seven experiments for NA concentrations in the range 1–20 μM (7.0 ± 6.3 μM). In most cases the restoration of the rhythm by NA lasted for the duration of the NA application (10–15 min), although in one case it only lasted for ~3–4 min, after which the rhythm broke down again.

**Discussion**

In this study we have shown that in the neonatal rat spinal cord, NA can trigger either tonic activity or a rhythmic motor pattern that is dominated by coactivation in ventral roots. NA also has a robust slowing effect on ongoing locomotor rhythms even at concentrations that are insufficient on their own to trigger any motor output. In addition, the NMDA/5-HT–induced rhythms can deteriorate into tonic activity after prolonged drug exposure, yet a robust and slower rhythm would resume after introduction of NA. Together these observations suggest that NA may be an important modulator of mammalian locomotor activity.
NA activation and modulation of locomotor activity

The rhythmic motor pattern evoked by higher concentrations of NA in the neonatal rat involves coactivation of functionally antagonistic motor roots and thus differs from the more regular alternating locomotor activity seen in adult spinal mammals after injection of the catecholamine precursor L-dihydroxyphenylalanine (L-DOPA) (Grillner and Zangger 1979; Jankowska et al. 1967; Viala and Buser 1969) or clonidine (Barbeau and Rossignol et al. 1998). Interestingly, the slowing effect occurred in the neonatal rat induced by 5-HT, glutamate-receptor agonists (Cazalets et al. 1990, 1992; Cowley and Schmidt 1994; Kiehn and Kjaerulff 1996; Kudo and Yamada 1987). However, the NA-induced rhythm bears some resemblance to the synchronous activity evoked by acetylcholine (Cowley and Schmidt 1994) and by strychnine in combination with bicuculline (Bracci et al. 1996; Cowley and Schmidt 1995).

Because the high degree of synchrony together with the relatively long cycle periods of the NA-induced rhythm are not well suited to perform normal locomotion, it seems more likely that the main role of NA in the neonatal rat is to modulate ongoing motor activity. In this respect the slowing of the locomotor rhythm by NA, accompanied by an increase in burst durations, with little effect on other locomotor parameters, is similar to the effects previously described in the cat (Rossignol et al. 1998). Interestingly, the slowing effect occurred without obvious changes in the coordination of the ongoing 5-HT/NMDA–induced locomotor activity, despite the fact that NA alone could produce a slow synchronous motor pattern very distinct from the 5-HT/NMDA rhythm. Similarly, when NA rescued the 5-HT/NMDA rhythm following its time-dependent rundown, this rhythm always reverted to a pattern similar in its coordination to the one present before the rundown. Furthermore, a characteristic of this modulatory effect of NA is that the rescued rhythm was always slower than the 5-HT/NMDA rhythm observed before deterioration. So it appears that when there is an ongoing rhythm in the presence of 5-HT/NMDA, the initiating role of NA is disguised by its modulatory slowing role.

The observation that NA can rescue rhythmic activity after prolonged exposure to 5-HT and NMDA may be useful for future experimental studies on the neonatal rat because it allows for longer-lasting stable locomotor activity. More importantly, this observation also raises the possibility that endogenously released NA, possibly originating from neurons in the locus coeruleus, could play a role in sustaining locomotor rhythm generation in the intact animal.

Mechanisms for the NA modulation

In the Xenopus tadpole, NA causes a slowing of the cycle period during swimming, with little effect on the burst durations (McDearmid et al. 1997) but a pronounced effect on the longitudinal coordination of the motor output (J. McDearmid, O. Kjaerulff, O. Kiehn, C. A. Reith, and K. T. Sillar, unpublished observations). At the cellular level, NA increases the amplitude of reciprocal, mid-cycle inhibition by enhancing glycinergic transmission in the network through a direct presynaptic facilitation of the release mechanism (McDearmid et al. 1997). It has therefore been proposed that NA slows the swimming rhythm by prolonging the inhibitory phase of the swimming cycle and so delaying the burst onsets (McDearmid et al. 1997). It is conceivable that a similar enhancement of reciprocal glycinergic transmission, which is known to contribute to spinal rhythm generation in the neonatal rat (Cowley and Schmidt 1995), could contribute to the slowing effect of NA on the locomotor rhythm. However, two observations suggest that additional effects of NA on the spinal networks are likely to be involved. First, in the absence of 5-HT/NMDA, NA can trigger both tonic and slow rhythmic activity, indicating an excitatory influence on spinal cord neurons. Second, the NA-induced slowing of the 5-HT/NMDA rhythm is not accompanied by any change in the amplitude of interburst activity, as one would predict to result from enhanced reciprocal inhibition (cf. McDearmid et al. 1997). These and other effects of NA on rhythm-generating elements in the network await exploration using intracellular recordings from motor and interneurons.
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