Endogenous Tachykinin Release Contributes to the Locomotor Activity in Lamprey

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INTRODUCTION

Despite the great diversity and variety in morphology within the animal kingdom, the literature abounds in examples of a common principle for motility in a broad range of phyla, namely that of locomotor generation by central pattern generators (CPGs). CPGs are local neuronal circuits responsible for the coordination of the different muscles generating propulsion (Arshavsky et al. 1998; Grillner 1974, 1975; Kiehn and Kjaerulff 1998; Nusbaum and Beenakker 2002). The lamprey serves as a simple vertebrate model system for the neural control of motor behavior. Swimming results from the activation of excitatory glutamatergic interneurons responsible for burst generation and inhibitory glycnergic neurons responsible for alternation (Buchanan and Grillner 1987; Cangiano and Grillner 2003, 2005; Cohen and Harris-Warrick 1984; Grillner et al. 2000).

In addition, several modulatory systems influence the locomotor network activity by modifying cellular and synaptic properties. 5-Hydroxytryptamine (5-HT) and dopamine (DA) are among the most studied neuromodulators in lamprey (Franck et al. 1992; Harris-Warrick and Cohen 1985; Kemnitz 1997; Svensson et al. 2003; Wallén et al. 1989; Zhang et al. 1996). Both monoamines are found in cells in the ventromedial spinal cord, which form a dense plexus into which spinal neurons extend their dendrites (Fig. 1A) (Schotland et al. 1995). These cells, which are active during locomotion (Christenson et al. 1989; Zhang and Grillner 2000), contain 5-HT and DA (Schotland et al. 1995), and a proportion also contain tachykinins (Auclair et al. 2004; Van Dongen et al. 1985, 1986).

Tachykinins (TKs) constitute a family of small neuropeptides (~11 amino acids) widely represented and distributed in the central and peripheral nervous system of chordates including the lamprey (Fried et al. 1988; Quartara and Maggi 1997). The functionally important sequence at the COOH-termini of the peptides has been conserved during vertebrate evolution (Waugh et al. 1995). Tachykinins bind to NK receptor subtypes and the NK receptors are G-coupled proteins (Maggi and Schwartz 1997; Quartara and Maggi 1997).

Substance P was the first tachykinin discovered (von Euler and Gaddum 1931), and it is a preferred ligand for the NK1 receptor, which is the most widely distributed TK receptor type (Maggi and Schwartz 1997). NK1 is known to activate both the adenylate cyclase and phospholipase C (PLC) second messenger systems (Quartara and Maggi 1997) and influence both the sensory and motor systems in the spinal cord (Cullheim and Arvidsson 1995; Hökfelt et al. 2001; Jacobs and Fornal 1997; Waugh et al. 1995).

Substance P–like peptides have been found in cells and fibers of the lamprey spinal cord, some of which contain 5-HT as well (Auclair et al. 2004; Van Dongen et al. 1985, 1986). This co-localization in the ventromedial plexus of the spinal cord suggests that it could also be released during locomotion to modulate the network activity. Previous studies show that exogenously applied TKs in lamprey and the neonatal rat elicit a prominent modulation of the frequency and regularity of the locomotor activity (Barthe and Clarac 1997; Parker et al. 1998). Moreover, the TKs elicit several effects on the cellular level including a protein kinase C–mediated potentiation of the N-methyl-d-aspartate (NMDA) component of glutamergic
synaptic transmission, accounting for part of the TK-induced effects (Parker and Grillner 1998, 2000; Parker et al. 1998). The main aim of this study is to explore whether an endogenous release of tachykinins contributes to the level of locomotor activity.

The results, which include an extensive analysis of the induction process of the progressive development of NMDA-induced fictive locomotion (burst frequency and regulation), suggest that an endogenous release of TKs contributes to the baseline frequency and to the initiation of locomotor activity. They further suggest that TKs are involved in maintaining a given activity level over time. The effects of an exogenous application of substance P were also analyzed. Part of these results has been reported in abstract form (Thörn Pérez et al. 2005).

METHODS

Intact spinal cords from 73 adult lampreys from two species were used. Lampetra fluviatilis were collected in Ljusne, Sweden, and Ichthyomyzon unicuspis was obtained from Iowa. They were kept in separate aerated aquaria at a temperature of 5°C. All protocols were approved by the Animal Research Ethical Committee, Stockholm. Lampreys were anesthetized with tricine methanesulphonate (MS 222, 100 mg/l; Sigma), and the preparation consisted of the glycogen-containing tissue layer surrounding the spinal cord and notochord were removed. The spinal cord and notochord were pinned to a Sylgard-lined chamber and continuously perfused with physiological solution at 8–10°C. The physiological solution to reach the final concentration (1 mM) was either stored frozen in saline with 0.01 M acetic acid (10 mM), or 1-mg units were directly dissolved into the physiological solution to obtain the desired concentration (4 μM). The acetic acid of itself at this concentration had no effect.

Pharmacology

Fictive swimming was induced by adding NMDA (Tocris, Bristol, UK) to the solution. A concentrated stock was kept frozen and during experiments was diluted to the final concentration (30–150 μM). Burst frequency increased gradually and stabilized after ≥3 h of NMDA perfusion (Fig. 1C), thereafter allowing long-term recordings (>20 h). Agonists and antagonists were added only after the burst frequency was stable. Frozen aliquots of substance P (1 mM; Sigma-Aldrich, St. Louis, MO) in water with 0.05 M acetic acid to prevent oxidation and 1% bovine serum albumin to increase the solution stability were stored at −20°C. They were dissolved in sufficient physiological solution to reach the final concentration (1 μM, physiological concentration range reported for neuropeptides by Duggan 1995) and applied for 10 min. Spantide II (Bachem, Rhein, Germany) was either stored frozen in saline with 0.01 M acetic acid (10 mM), or 1-mg units were directly dissolved into the physiological solution to obtain the desired concentration (4 μM). The acetic acid of itself at this concentration had no effect.

Stock solutions of sendide (Bachem, Rhein, Germany), RP–678 (Tocris) and L-72338 (Sigma-Aldrich) were made by dissolving them in ethanol and kept at room temperature.

Statistics

One-minute recordings of ventral root activity were sampled every 20 min. The CV (CV = SD/average × 100) was taken as the measure of regularity; summary statistics are reported as SD, and P values were calculated using Student’s t-test.

RESULTS

Although it is commonly known that the development of stable NMDA-induced fictive locomotor activity requires some time, this important aspect has not been analyzed in any detail in the lamprey. Physiological changes, modulatory effects, or other perturbations of the rhythm can only be assessed when the rhythm has stabilized. We therefore provide a detailed description (frequency and regularity) of the progressive development of NMDA-induced fictive locomotion (L. fluviatilis) to be able to explore a possible role of TKs in the locomotor network.
Stable baseline locomotor frequency requires long adaptation time and is concentration dependent

Application of NMDA to the spinal cord evokes fictive locomotion. Within 10 min, after the addition of 50 μM NMDA, the quiescent spinal cord starts to elicit burst activity, and in the following period lasting ~20 min, the burst pattern is often very irregular, and one side may dominate with long bursts. Figure 1C shows the irregular activity at minute 20 followed by more regular activity at 80 and 160 min of 50 μM NMDA application. The locomotor rhythm develops gradually (Fig. 1C2), and the frequency increases markedly (71%) from 0.4 to 1.4 Hz during 160 min of NMDA application (Fig. 1C3). The locomotor burst frequency induced by different NMDA concentrations (50-75-100 μM) was analyzed. Figure 2A shows the frequency versus time for three NMDA concentrations in different pieces of spinal cord of one animal. A pronounced increase in frequency occurs during the first 2 h followed by a smaller change during the next few hours, and eventually a stable burst frequency is reached. We defined the stabilizing period as the time it takes to reach a stable pattern of activity, which is when the average change in frequency is not more than ±5% over a period of 40 min. At higher concentrations, the stabilizing period is shorter and a higher frequency is reached. Figure 2B shows the average and SD of all spinal cords tested with 50 (n = 5), 75 (n = 5), and 100 μM (n = 5) NMDA. All experiments follow a similar trend as the one shown in Fig. 2A, and all were consistent in developing the rhythmic activity, but varied in regularity and alternation, particularly during the first hour. As a measure of regularity of the burst frequency, the CV was calculated during 1 min every 20 min. In Fig. 2C, the averaged CV of preparations represented in Fig. 2B is shown for different levels of NMDA. Initially, the CV is the highest at the lowest NMDA concentration, but a decrease in the burst variation occurs over the first few hours, indicating that the rhythmic pattern becomes more regular over time at all concentrations. At 40 min of NMDA perfusion (Fig. 2C), the CV was significantly different (P < 0.01) between 50 μM (SD = 17.8) and the higher concentrations (75 μM, SD = 17.7; 100 μM, SD = 9.7), and later it approached the same level of variability as with higher NMDA levels.

After the progressive increase in frequency during the first hours of locomotion, a maximum frequency, dependent on the NMDA concentration, is reached. The maximum frequency was defined as the frequency reached after 5 h of NMDA perfusion because, after this time, no significant change in frequency occurs. Figure 2D shows the maximum frequency based on the average of all experiments at four given concentrations of NMDA. Different pieces of spinal cord were used for each experiment. NMDA induced significantly higher maximum frequencies with higher concentrations (100 μM, SD = 0.39; 150 μM, SD = 0.3). As a measure of the stabilizing period, we used the time it takes to reach one half of the maximum frequency (half time) because it was easier to define. Figure 2E shows the relation between the maximum frequency and the half time at three different NMDA concentrations. For each NMDA concentration, the half time was inversely related to the concentration, taking only 37 ± 9.2 min at 100 μM but 69 ± 19 min at 50 μM. The time to half-maximum frequency was significantly shorter for 100 μM of NMDA than for the lower concentrations. There is thus a pronounced effect on the rate of burst frequency adaptation with NMDA concentration.

Ichthyomyzon unicuspis, a North American species, has also been used extensively in locomotor studies (Grillner et al. 1981); we therefore compared this species with L. fluviatilis. The time to half-maximum frequency required for I. unicuspis (Fig. 2F) was significantly shorter than the time required for L.

![Figure 2](http://jn.physiology.org/ by 10.220.33.1 on August 28, 2017)
fluviatilis with 75 and 100 μM NMDA (P < 0.05). The frequencies at different concentrations of NMDA were comparable in both species.

Thus the results in Fig. 2 show that, at low concentrations of NMDA (<75 μM), a slower acceleration occurs such that more time is needed to reach a stable burst frequency. At higher concentrations, the burst frequency develops faster. The time necessary to reach a stable frequency for a given concentration must be considered in experiments that require a control baseline frequency of fictive locomotion.

Tachykinins—endogenous effect

To test if tachykinins are released endogenously during fictive locomotion and, if so, to what extent they contribute to the locomotor activity, 4 μM of spantide II, a competitive antagonist for substance P that specifically binds to NK1 receptors (Hakanson et al. 1990; Kikwai et al. 2004), was applied during stable fictive locomotion. It has previously been shown to antagonize the effect of tachykinins in the lamprey spinal cord at the sensory, interneuron, and motoneuron levels (Parker and Grillner 1996; Parker et al. 1998; Svensson et al. 2002).

Figure 3A shows that stable ventral root bursting elicited by 100 μM NMDA (control) was reduced from 1.8 ± 0.3 to 1.4 ± 0.3 Hz (17%) after 1 h of spantide II application. Figure 3B shows an experiment in which two pieces of spinal cord from the same animal were placed in separate chambers: one initially in physiological solution (squares) and the other in spantide II (circles), for 1 h. NMDA was added to both, and the spantide II concentration was maintained for the preincubated piece. In the latter piece, the rhythm was initially irregular (cf. Fig. 1C) to stabilize at a lower frequency than in the control. The untreated spinal cord thus developed a higher frequency than that with the TK antagonist. Moreover, when spantide II was applied to the control spinal cord, its frequency was reduced to the same level as that of the preincubated piece. The average of burst frequency over time for six experiments is shown in Fig. 3C, indicating an ~20% decrease. Figure 3D provides a summary of the burst frequency decreases in individual experiments caused by spantide II over 4 h of application (n = 6). These data clearly indicate that an endogenous release of TKs contributes to the overall frequency.

Spantide II, considered to be an NK1 antagonist in mammals, led to a significant reduction in the burst frequency (Fig. 3D) in five of six cases. The mammalian NK1 blocker sendide (4–10 μM) also caused a significant reduction of burst frequency in two of five experiments, and in the other three, a small reduction was encountered. L-72338 (4–10 μM), yet another mammalian NK1 blocker, had no detectable effect on fictive swimming (n = 3; data not shown).

Long-lasting plastic changes occur during NMDA-induced fictive locomotion to which TKs contribute

Because we observed that the adaptation period after NMDA application lasts up to several hours, we explored if the gradual increase in frequency and stability might be caused by a progressive action of modulatory factors such as tachykinins. To test this, we first measured the time to half-maximum frequency, as described above. We washed out NMDA for 1 h and repeated the measurements after NMDA application. Figure 4A1 shows the average time-course for six experiments where the time to half-maximum frequency is shown in the shaded area for the first and second application. This value was significantly shorter (P < 0.005) for the second application (Fig. 4A2) (50 vs. 10 min). This suggests that a plastic change had been induced during fictive locomotion, an action that remained for ≥1 h. The effect on locomotion induced by N-glutamate activating both NMDA and AMPA receptors was also tested (n = 3). The results were similar to those reported for NMDA (data not shown).

To study whether TKs contributed to these changes, spantide II was applied starting at the onset of washout in eight experiments (Fig. 4B1). The time to half-maximum frequency was similar and not significantly different (P > 0.05) from that of the first NMDA application (Fig. 4B2; 50 vs. 30 min). As expected, because of the blockade of TK receptors, the mean stabilized frequency was also somewhat reduced, but not significantly. These results support the notion that endogenously released TKs contribute to the plastic changes occurring during NMDA application.

**FIG. 3.** Endogenous release of tachykinins during locomotion. A: stable ventral root bursting induced by 100 μM NMDA (control) and VR bursting after 1 h of blockade of tachykinins receptors by the antagonist spantide II (4 μM) that decreased frequency by ~17%. B: a control piece of spinal cord developed a stable rhythm with 75 μM of NMDA (squares). Another piece of spinal cord from the same animal was treated with spantide II 1 h before addition of NMDA (circles). The treated piece had a lower frequency than the control. When spantide II was applied to the control spinal cord, its frequency was reduced to a similar level as the treated one. C: plot of averaged data from 6 different preparations showing time-course of decrease in locomotor burst frequency. Burst frequency decreased ~20% after 5 h of spantide II application. Error bars show SD. D: summary of data from 6 experiments where effect of spantide II was measured before and after 3 h (***P < 0.05; **P < 0.01; +++P < 0.005 when comparing mean cycle duration for 20 cycles).

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Substance P has both short- and long-term effects on the locomotor network

Brief activation of tachykinin receptors by exogenous application (10 min) has been reported to have a long-term effect on the locomotor burst frequency (Parker et al. 1998). Because we observed the outcome of TK receptor blockade, we were interested in analyzing the short-term effects of exogenous substance P on the locomotor burst frequency during relatively low and high burst rates, respectively.

A 10-min application of substance P (1 μM) during fictive locomotion induced by 30 μM NMDA caused a marked slowing in frequency. Figure 5, A–C, shows the ventral root recording of a single experiment where the fictive locomotion induced by 30 μM of NMDA is slowed from 1 to 0.2 Hz with substance P application. The slow rhythm replaced the initial faster rate of bursting for 1 h, and during the second hour of washout gradually recovered to reach a somewhat higher frequency than before the substance P application (1.3 Hz; Fig. 5C). With a higher stable burst frequency induced by 50 μM NMDA (1.38 Hz; Fig. 5D), a 10-min application of substance P (1 μM) caused an enhanced burst frequency, superimposed on a much slower modulation of the amplitude of the burst activity (0.1 Hz; Fig. 5E), followed by a recovery of a regular burst pattern at an increased frequency during washout (1.7 Hz; Fig. 5F).

Figure 6A shows the time-course of the frequency changes for the ventral root recordings of Fig. 5, A–C and also reveals a significant but small long-lasting frequency increase (P < 0.005). The burst frequency change is also shown as a power spectrum function (Fig. 6B) that shows the change to a slow frequency and an increase in burst rate after the washout. The long-term effect was tested in three preparations, and it lasted for as long as the preparation was observed (>3 h).

FIG. 4. Adaptation time to half-maximum bursting frequency of a 2nd NMDA application and role of TKs. A1: 2nd exposure to the same concentration of NMDA (50 μM), after 1 h of washout, required shorter mean time (80% less) to reach the maximum frequency in 6 experiments. A2: graph showing time to half-maximum frequency of the 1st and the 2nd application of NMDA (P < 0.001; n = 6). B1: 2nd exposure to the same concentration of NMDA (plus spantide II), after 1 h of spantide II preincubation in 6 experiments, required similar time to reach the initial time to the half-maximum frequency (gray area, n = 8). B2: graph showing time to half-maximum frequency of 1st application of NMDA and 2nd application of NMDA with spantide II, which was not significant (P > 0.39; n = 8). Error bars show SD.

FIG. 5. Activation of tachykinin receptors has an acute effect on locomotor burst frequency. A–C: 10-min application of substance P (1 μM) during fictive locomotion induced by 30 μM of NMDA caused a slow rhythm to appear, replacing the initial rate of bursting. A single ventral root recording shows the control, substance P short-term effect, and washout, showing effects at the same time scale and a longer one to show rhythm. D and E: 10-min application of substance P (1 μM) during fictive locomotion induced by 50 μM of NMDA imposed a slower rhythm on the fast one. A single VR recording shows the control, substance P short-term effect, and washout, again showing effect with 2 time scales.
A 10-min application of substance P (1 μM) during fictive locomotion induced by 30 μM NMDA \((n = 3)\) thus had a short-term effect, which induced a much slower burst pattern, and after washout, a minor long-term frequency increase occurred.

The time-course of the 50-μM experiment shown in Fig. 5, D–F is shown in Fig. 6D (slow and fast bursting). The power spectrum (Fig. 6E) shows the induction of the low frequency and the higher frequency. The higher frequency persisted after the washout. A fast and superimposed low burst pattern occurred also with 100 μM NMDA (Fig. 6F). The slow modulation of the amplitude of the motor activity persisted for 1–2 h after washout.

The increase in frequency caused by substance P application is summarized in Fig. 6C for seven experiments at two different NMDA concentrations. It shows that a brief application of substance P (10 min) caused an increase in frequency lasting for \(\geq 3\) h as shown in the examples of Fig. 5, A and D, compared with Fig. 5, C and F, respectively. This significant but modest long-term effect occurred in six of seven cases for both low and higher burst rates at different NMDA concentrations.

Seasonal variation has been reported with regard to the quality of the burst pattern induced by NMDA (Cangiano 2004), as estimated from autocorrelograms (Cangiano and Grillner 2003), but not on the burst frequency itself. We therefore compared the effect of substance P in fall and spring, and no consistent changes were observed (data not shown). Note that the CV was markedly reduced during the first hours of NMDA application (Fig. 2C). Spontaneous II application affected the regularity and strength of the rhythmic bursting in some cases, but no consistent changes were observed.

During the initial adaptation period, before a stable burst frequency had been reached, it was pertinent to ask if substance P also affected the burst frequency. Figure 7 shows a single experiment in which two pieces from the same animal were tested. The control is a recording of the fictive locomotion induced by 100 μM of NMDA during 200 min. In the test piece, substance P was applied for 10 min at minute 70 after NMDA application. The graph shows that the burst frequency is significantly enhanced over the control \((P < 0.001)\). Superimposed on this faster rhythm, a slow modulation of the amplitude of the motor activity is apparent as also shown in Fig. 6F during steady-state application of substance P. Substance P applied at 20 \((n = 3)\) or 70 \((n = 3)\) min after the onset of NMDA bath application elicited a significant increase \((P < 0.005)\) over that of the controls in five of six cases. Even though no difference was noticed when both pieces had the same conditions, rostral and caudal pieces alternated as controls in these experiments.

Taken together, these results indicate two main effects of a 10-min application of substance P (1 μM) when a steady-state frequency had been reached: a short-term effect of a low-frequency component that is dependent on burst frequency or NMDA concentration and a second, modest long-lasting effect \((\geq 3\) h) that was not. A 10-min application of substance P (1 μM) when the frequency had not yet stabilized also revealed a sharper rise of the burst frequency during NMDA application (Fig. 7).
TACHYKININS AND LOCOMOTOR PATTERN GENERATION

DISCUSSION

Adaptation

The results of this study entail a detailed description of the progressive development of NMDA-induced fictive locomotion with regard to burst frequency and regularity. This was important because a stable baseline is needed for the analysis of the effects of TKs. NMDA evokes fictive locomotion with a burst frequency ranging from ~0.8 to 3.5 Hz (Wallén and Williams 1984). In L. fluviatilis, ≥2 h are needed for the rhythm to achieve stability. Thus some changes must occur in the network function during this long period of adaptation. One likely explanation is provided by metabotropic receptors, activated by an endogenous release of transmitters acting through protein phosphorylation. For example, an endogenous activation of metabotropic glutamate receptors (mGlurRs) has been shown to modulate the release of transmitter and to contribute to the baseline frequency through different cellular mechanisms (Cochilla and Alford 1998; Kettunen et al. 2005; Krieger et al. 1994). In these experiments, the decrease in burst frequency after application of the TK receptor antagonist, spantide II, indeed indicated that an endogenous release of TKs also plays a role in setting the baseline frequency.

The network effects induced after several hours of NMDA application, which resulted in a shorter time to reach a stable rhythm, lasted for ≥1 h after washout—thus a sort of “memory” effect was present (Fig. 4). Therefore some changes must have taken place, which tended to stabilize the CPG activity in a particular state. Studies involving experimental changes in NMDA or glutamate concentrations before reaching a stabilized frequency for the given conditions cannot be compared directly to these data and could therefore be misleading. To achieve a more accurate estimate of the maximum frequency for a given NMDA concentration, it was thus necessary to repeat the measurements and to use different pieces of spinal cords from the same animal. Moreover, when the spinal cord was preincubated in the tachykinin antagonist spantide II, applied during the NMDA application, the long-term frequency adaptation was not seen (Fig. 4B), which, in addition to the frequency decline induced by spantide II during stable swimming, suggests that TKs contribute importantly to potentiating the level of locomotor activity.

The results involving changes in frequency during the NMDA induction of locomotor activity must be taken into account in future experiments, particularly in L. fluviatilis, because a constant baseline is essential when evaluating the application of any drug. The origin of the species differences observed here between L. fluviatilis and I. unicuspis (Fig. 2F) in the level of NMDA adaptation is as yet unclear, but could possibly be related to differences in behavioral requirements between the two species, one of which (L. fluviatilis) is migratory.

Endogenous release of TKs

Modulation of the locomotor frequency occurs at the level of the spinal cord, enabling both fast responses to sudden demands during locomotion, and fine tuning of the activity of the locomotor network to meet long-term behavioral demands (Grillner 2003). Immunohistochemistry has been a helpful tool in identifying potential neuromodulators and in studying both aminergic and peptidergic modulatory systems in the lamprey (Harris-Warrick and Cohen 1985; Wallén et al. 1989; Wikstrom et al. 1999). 5-HT, DA, and tachykinins have been observed in cell bodies and branches of the plexus below the central canal (Van Dongen et al. 1985). Tachykinins have been shown to modulate the locomotor network (Parker et al. 1998), but there has been no clear evidence of an endogenous release of TKs acting on the locomotor network. This study provides evidence for the release of TKs during fictive locomotion and that it contributes to the baseline frequency. Thus an application of the TK NK₁ antagonist, spantide II, decreased the locomotor burst frequency, suggesting that there is indeed an endogenous release of TKs. This action may also be reproduced by sendide, another NK₁ like receptor agent. Candidate cells involved in the release of the substance P–like peptides are located in the ventromedial plexus that have been shown to contain 5-HT and tachykinins (Auclair et al. 2004; Van Dongen et al. 1985) and that co-localize 5-HT and DA (Schotland et al. 1995). TKs are stored in large dense-core vesicles and can be co-localized with 5-HT (Pelletier et al. 1981; Van Dongen et al. 1985). No synaptic specializations have been found in the ventral plexus (Christenson et al. 1990), and therefore, 5-HT and TKs are thought to be released paracrinically from varicosities and act on surrounding dendrites of network neurons.

Effect of exogenously applied substance P

Van Dongen et al. (1985, 1986) showed that spinal neurons and fibers contain TK immunoreactivity in three different patterns in the adult L. fluviatilis and I. unicuspis. Auclair et al. (2004) concluded that at least two different TKs are present in the spinal cord of newly transformed Petromyzon marinus, corroborating the presence of these peptides. A number of immunohistochemical studies suggest that the distribution of tachykinins in the CNS is similar in vertebrates (Kar and
Quirion 1995). The NK₁ receptor subtype seems to be preserved and increase in density during evolution, whereas the NK₂ type is more abundant in lower vertebrates and apparently absent in primates. NK₂ receptors are absent in the vertebrate CNS (Dietl and Palacios 1991). An exogenous application of substance P (1 μM) to the spinal cord of neonatal rat and lamprey modulates the locomotor network by increasing the frequency and improving the regularity of the burst activity (Barthe and Clarac 1997; Parker et al. 1998). A confounding factor, as shown here, is that the rhythm becomes more stable after several hours of NMDA perfusion. After achieving a stable rhythm, we found no further effect of substance P on the regularity. When substance P (1 μM) was applied at different NMDA concentrations, two different short-term effects (≤1 h) were observed. At low initial frequencies (<0.8 Hz) and low NMDA concentrations, substance P induced very slow alternating bursts (~0.1 Hz). With higher initial frequencies (>1 Hz) and higher NMDA concentrations, substance P instead induced a faster burst rate (1.5 Hz) in which there was a superimposed slow burst modulation (0.1 Hz).

The very slow bursting is dependent on NMDA receptors and their voltage dependence (Brodin and Grillner 1986) and can be observed in the hemicord preparation (Cangiano and Grillner 2003) and also in the neonatal rat (Gabbay and Lev-Tov 2004). This pattern is characteristic of NMDA-elicited locomotion, which at the cellular level induces plateau properties and oscillations (Wällén and Grillner 1987). From this it follows that the slow motor pattern probably is linked to NMDA plateau properties. Substance P is known to enhance the NMDA current in lamprey neurons (Parker et al. 1998), an effect that presumably can account for the switch from stable low initial frequency (<0.8 Hz) to a radically lower burst rate (~0.1 Hz). These bursts are alternating between the right and left sides, but the frequency elicited is slower than that of regular locomotion. This slow activity might instead correspond to motor patterns such as struggling.

The fast motor pattern is firmly linked to swimming, because it is similar to that seen under control conditions (Cangiano and Grillner 2003; Wällén and Williams 1984). In the spinal cord of the neonatal rat, an exogenous application of substance P increased the burst rate recorded from ventral roots (Barthe and Clarac 1997). Furthermore, this activity progressively disappears after 10 min of substance P application. In the lamprey spinal cord, application of substance P has been reported to increase the frequency and cause a long-term response (Parker et al. 1998). Our results show that, in most cases, a long-lasting increase the frequency and cause a long-term response (Parker et al. 1998). With higher initial frequencies (>0.8 Hz) and low NMDA concentrations, substance P induced a faster burst rate (1.5 Hz) in which there was a superimposed slow burst modulation (0.1 Hz). With higher initial frequencies (>1 Hz) and higher NMDA concentrations, substance P instead induced a faster burst rate (1.5 Hz) in which there was a superimposed slow burst modulation (0.1 Hz).

In conclusion, our results indicate that there is an endogenous release of TKs during locomotor activity and that TKs contribute to increased burst frequency. They also indicate that TKs provide plastic changes occurring at the network level that last over an hour. Substance P application can give rise to short-term induction of a slow rhythm and a long-term effect of a modest increase in frequency.

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