Short communication

The spinal 5-HT system contributes to the generation of fictive locomotion in lamprey

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Abstract

Activation of NMDA receptors evokes sustained fictive locomotion in the isolated spinal cord of the sea lamprey \textit{Petromyzon marinus} (\textit{P. marinus}), but in the river lamprey \textit{Lampetra fluviatilis} (\textit{L. fluviatilis}) the ventral root activity is often irregular. A previous study showed that the number of 5-HT immunoreactive fibres, neurones and varicosities are much lower in the spinal cord of \textit{L. fluviatilis} than in \textit{P. marinus}. To further analyse the underlying mechanisms, the present study investigated the role of the 5-HT system in stabilising fictive locomotion. In \textit{P. marinus} a blockade of 5-HT\textsubscript{1A} receptors by spiperone reversibly increased the frequency and the coefficient of variation. This implies that there is an endogenous release of 5-HT during fictive locomotion that is important for the generation of locomotor activity. In \textit{L. fluviatilis} bath applied NMDA or D-glutamate evoked in most cases irregular activity. An addition of 5-HT (0.5–2 \textmu M) rapidly stabilised the burst generation and led to a sustained fictive locomotion. In a split-bath configuration, NMDA administered to the rostral part of the spinal cord in \textit{P. marinus} evoked fictive locomotion in both the rostral part and the first few segments of the caudal part. When spiperone was added to the caudal part, the burst activity changed into tonic activity within 10 min. Taken together, these results indicate that activity in the intrinsic 5-HT system in the lamprey spinal locomotor network contributes significantly to the rhythm generation. The quantitative differences with regard to the 5-HT plexus between \textit{P. marinus} and \textit{L. fluviatilis} may account for the observed discrepancy between the two species. © 2000 Elsevier Science B.V. All rights reserved.

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5-hydroxytryptamine (5-HT) exerts a profound influence on the locomotor pattern generation in a wide range of vertebrates [2,9,12,15,16]. In the lamprey spinal cord, the processes of intraspinal 5-HT neurones form a dense ventromedial plexus [11,17] with 5-HT varicosities. These neurones appear to release 5-HT in a paracrine fashion [6], resulting in a modulation of locomotor interneurones and motor neurones [13,17]. The effect can be accounted for by a decreased conductance of calcium-dependent potassium channels (\textit{K}\textsubscript{Ca}) [18,19], although other actions of 5-HT may also contribute [5,14,19]. In the isolated spinal cords of the adult silver lamprey \textit{Ichthyomyzon unicuspis} (\textit{I. unicuspis}) and sea lamprey \textit{Petromyzon marinus} (\textit{P. marinus}) rhythmic locomotor activity can be elicited by application of excitatory amino acid agonists like NMDA, AMPA, kainate or \textit{n}-glutamate [1,3,4,7,10]. In the river lamprey \textit{Lampetra fluviatilis} (\textit{L. fluviatilis}), on the other hand, an application of excitatory amino acids agonists will in many cases evoke tonic activity from ventral roots without a clear reciprocal burst activity [7]. A quantitative comparison between the size of the 5-HT plexus in \textit{P. marinus} and \textit{L. fluviatilis} has shown that it is much better developed in the former species [20], a finding which

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could account for the difference in locomotor co-ordination. The present study investigates the role of the 5-HT system in the control of locomotion in these two species.

Isolated spinal cord preparations of river lampreys _L. fluviatilis_ (n=20), and sea lampreys _P. marinus_ (n=20) were analysed. Fictive locomotion was induced with NMDA (cf. [10]) or _d_-glutamate (cf. [7]) in a Ringer solution with the following compositions (in mM): NaCl 138, KCl 2.1, CaCl$_2$ 1.8, MgCl$_2$ 1.2, Glucose 4, Hepes 2 and _l_-glutamine 0.5. Locomotor activity was recorded from the ventral roots with glass suction electrodes. Under control conditions, the concentrations of NMDA and _d_-glutamate were gradually increased from 20 to 250 _µM_ and from 250 to 1000 _µM_, respectively. All drugs were administrated by bath application, and the effects of different drug concentrations were tested 10 min after drug application. In some experiments (cf. Fig. 3), rostral and caudal segments of the spinal cord were separately perfused with different bath solutions in a chamber that was divided by a plastic barrier sealed with Vaseline. Before each experiment, the seal of the plastic barrier was carefully tested using a small amount of Fast Green FCF (Sigma). During the experiments, each pool was continuously perfused with the desired solution (cf. [8]). The cycle duration (in seconds) of the locomotor rhythm was measured between the mid-burst of successive ventral root discharges. The instantaneous frequency in Hz was calculated as the reciprocal of the cycle duration. The coefficient of variation was defined as the percentage of the standard deviation divided by the instantaneous frequency.

Unlike in vitro spinal cord preparations of the silver lamprey _I. unicuspis_ and the sea lamprey _P. marinus_, application of NMDA (50–250 _µM_) did as a rule not elicit regular burst activity in _L. fluviatilis_ (n=15, Fig. 1A, cf. also [7]). One minute after 5-HT (1 _µM_) was added to the NMDA-containing bath solution, a regular and alternating burst pattern emerged, which lasted as long as 5-HT was present (60 min, Fig. 1B). After application of spiperone (5 _µM_), a 5-HT1A receptor antagonist, the ventral roots activity became tonic (Fig. 1C), as before the application of 5-HT (cf. Fig. 1A). The same is true for _d_-glutamate-induced fictive locomotion (n=5, data not shown). Adding increasing levels of 5-HT (0.5–2.0 _µM_) decreased both the frequency and the coefficient of variation of burst activity (Fig. 1D, E). The diagrams in Fig. 1D, E summarise the effects 5-HT (0.5–2.0 _µM_) at different levels of NMDA.

**Fig. 1.** 5-HT improved NMDA induced locomotor burst generation in _L. fluviatilis_. (A) In the isolated spinal cord of _L. fluviatilis_ (n=15) bath application of 100 _µM_ NMDA only evoked tonic activity in ventral roots on the left (L) and right (R) sides of a segment. (B) After the application of 5-HT (1 _µM_) regular locomotor burst activity appeared. (C) This effect of 5-HT could be antagonised by application of spiperone (5 _µM_). (D, E) The 3D diagrams show the average of the normalised burst frequency (normalised to the highest burst rate at 200 _µM_ NMDA and 0.5 _µM_ 5-HT), and the coefficient of variation from five experiments at different NMDA (50–200 _µM_) and 5-HT (0.5–2 _µM_) levels. In these experiments, application of NMDA without 5-HT only elicited tonic activity.
(50–200 μM) on the frequency and the coefficient of variation (CV; n=5). At all NMDA levels (Fig. 1D) 5-HT causes a reduction of the burst frequency. The reduction (%-wise) is lowest at the highest burst frequencies/NMDA levels. Increasing levels of 5-HT causes a reduction in the CV and thus a more regular burst pattern. These results indicate that 5-HT can play a crucial role in the control of fictive locomotion.

In P. marinus (n=15), which has a more extensive 5-HT plexus than L. fluviatilis [20], NMDA induced a regular alternating burst activity in a dose-dependent manner without externally applied 5-HT (Fig. 2A, D cf. also [10]). The relation between NMDA concentration and burst frequency was S-shaped (Fig. 2D, filled symbols). In all experiments (n=15), the coefficient of variation of burst frequency was lower than 10% in the flat part, but increased up to 50% in the steep part of the dose–response curve (Fig. 2D, open symbols), although the burst activity was strictly alternating between the two sides of the spinal cord [14]. An addition of 1 μM 5-HT significantly reduced the frequency at different NMDA levels and prolonged the duration of burst activity (Fig. 2B), whereas the burst frequency was more regular with a lower coefficient of variation (compare Fig. 2D and E). Spiperone (10 μM), on the other hand, caused an increase of both the frequency (Fig. 2C) and the coefficient of variation of NMDA induced burst activity (Fig. 2F). The presence of 10 μM spiperone and NMDA (>150 μM) irreversibly (after 5-h washout) disrupted the rhythmic burst generation (asterisk in Fig. 2F). To test whether both the endogenous release and the effects of 5-HT on fictive locomotion were accompanied by activation of NMDA receptors, the rostral and caudal portions of the isolated spinal cord were separated (see inset Fig. 3) and perfused with different solutions (n=5, cf. [8]). When the rostral pool was perfused with NMDA (100 μM) containing Ringer solution, regular burst activity occurred both in the rostral and in the first few segments of the caudal pool (Fig. 3A). The burst activity in the caudal pool was rhythmic and alternating but the amplitude of the burst activity was often lower as compared to that in the rostral pool. After application of spiperone (10 μM) in the caudal pool, the activity pattern gradually became more tonic (Fig. 3B, lower trace, 5 min after drug application). In the rostral pool, both the frequency and the amplitude of the burst activity were maintained (Fig. 3B, upper trace). Upon washout of spiperone with Ringer solution in the caudal pool, rhythmic burst activity reappeared within 15 min (Fig. 3C). This result implies that a blockade of 5-HT receptors in the caudal pool affects burst generation only in the caudal pool, which, however, is driven from the rostral pool activating both interneurones and motoneurones near the barrier in the caudal pool. In this case 5-HT neurones within the caudal pool that is not subject to direct NMDA activation, and they must be activated synaptically, as a consequence of the drive from the rostral pool. In the case of Fig. 2, 5-HT neurones could behave been activated by the bath applied NMDA rather than synaptically.

The present effects of spiperone indicates that an endogenous release of 5-HT normally plays both a

![Fig. 2. Blockade of 5-HT1A receptors increased the frequency and the coefficient of variation of NMDA-induced fictive locomotion in P. marinus. (A–C) The traces show examples of recordings under control conditions (A), with 5-HT (1 μM, B) and spiperone (10 μM, C) as indicated. (D–F) Diagram showing the dose–dependency with the corresponding coefficient of variation (CV) curves of NMDA-induced fictive locomotion in P. marinus (n=15), under control (D), 5-HT (1 μM, E) and spiperone (10 μM, F). The dashed curve in E shows the dose–response curve under control (A), for comparison. Note that the presence of spiperone (10 μM) and NMDA (>150 μM) causes an irreversible brake down of the rhythmic burst activity (*) in F.](image)
The present study shows that the level of activity in 5-HT releasing neurones are an integral part of the spinal locomotor system. The role of 5-HT will be to modulate cellular properties like the after-hyperpolarisation of the pattern generating neurones [18], to be appropriate for generating the locomotor pattern, and possibly also to affect the synaptic interaction. A certain level of activity in a modulator system like the 5-HT system appears thus to be important.

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References

[4] L. Brodin, S. Grillner, C.M. Rovainen, N-Methyl-D-aspartate (NMDA), kainate and quisqualate receptors and the generation of modulatory and a stabilising role in the spinal locomotor network. The difficulty to evoke rhythmic motor output in the spinal cord of L. fluviatilis by application of excitatory amino acids could be accounted for by a smaller amount of endogenously released 5-HT due to the smaller 5-HT innervation in L. fluviatilis [20]. In support of this interpretation, the present study showed that external application of 5-HT and NMDA evoked dose-dependent fictive locomotion and decreased the coefficient of variation in L. fluviatilis (Fig. 1) that is quite similar to that in P. marinus (Fig. 2). External administration of 5-HT (0.5–2 μM) may thus mimic the effect of an endogenous release of 5-HT, which might not be sufficient in L. fluviatilis under experimental condition. Recent complementary experiments, using voltammetry, show that increased levels of 5-HT are released during fictive locomotion (Gonon, Wallén, Svensson and Grillner, unpubl.). This applies also to areas of the spinal cord, which are not subjected to bath application of NMDA but are driven by propriospinal interneurones (cf. Fig. 3). Also in this case a 5-HT receptor blockade with spiperone blocked the burst activity. This finding thus suggests that 5-HT neurones in this area release 5-HT either tonically or that they are driven by the propriospinal innervation from the rostral spinal cord in the split bath configuration.

Fig. 3. The effect of spiperone is not dependent on an activation of NMDA receptors. In double pool experiments (n=5), the rostral pool (a) was perfused with NMDA (100 μM) containing solution, while there was only Ringer solution in the caudal pool (b) in the control experiments (A). Rhythmic burst activity could be recorded in the first few spinal ventral roots of caudal pool (lower trace in A, cf. also [8]). (B) Application of spiperone (10 μM) in the caudal pool elicited tonic activity after 5 min (C). The effect of spiperone could be washed out upon perfusion of Ringer solution in the caudal pool. The inset shows the schema of the recording.


