Neuropeptide-Mediated Facilitation and Inhibition of Sensory Inputs and Spinal Cord Reflexes in the Lamprey

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Ullström, Maria, David Parker, Erik Svensson, and Sten Grillner. Neuropeptide-mediated facilitation and inhibition of sensory inputs and spinal cord reflexes in the lamprey. J. Neurophysiol. 81: 1730–1740, 1999. The effects of neuromodulators present in the dorsal horn [tachykinins, neuropeptide Y (NPY), bombesin, and GABA<sub>B</sub> agonists] were studied on reflex responses evoked by cutaneous stimulation in the lamprey. Reflex responses were elicited in an isolated spinal cord preparation by electrical stimulation of the attached tail fin. To be able to separate modulator-induced effects at the sensory level from that at the motor or premotor level, the spinal cord was separated into three pools with Vaseline barriers. The caudal pool contained the tail fin. Neuromodulators were added to this pool to modulate sensory inputs evoked by tail fin stimulation. The middle pool contained high divalent cation or low calcium Ringer to block polysynaptic transmission and thus limit the input to the rostral pool to that from ascending axons that project through the middle pool. Ascending inputs and reflex responses were monitored by making intracellular recordings from motor neurons and extracellular recordings from ventral roots in the rostral pool. The tachykinin neuropeptide substance P, which has previously been shown to potentiate sensory input at the cellular and synaptic levels, facilitated tail fin-evoked synaptic inputs to neurons in the rostral pool and concentration dependently facilitated rostral ventral root activity. Substance P also facilitated the modulatory effects of tail fin stimulation on ongoing locomotor activity in the rostral pool. In contrast, NPY and the GABA<sub>B</sub> receptor agonist baclofen, both of which have presynaptic inhibitory effects on sensory afferents, reduced the strength of ascending inputs and rostral ventral root responses. We also examined the effects of the neuropeptide bombesin, which is present in sensory axons, at the cellular, synaptic, and reflex levels. As with substance P, bombesin increased tail fin-stimulation-evoked inputs and ventral root responses in the rostral pool. These effects were associated with the increased excitability of slowly adapting mechanosensory neurons and the potentiation of glutamatergic synaptic inputs to spinobulbar neurons. These results show the possible behavioral relevance of neuropeptide-mediated modulation of sensory inputs at the cellular and synaptic levels. Given that the types and locations of neuropeptides in the dorsal spinal cord of the lamprey show strong homologies to that of higher vertebrates, these results are presumably relevant to other vertebrate systems.

INTRODUCTION

Although it is important to know the effects of neuromodulators at the cellular and synaptic levels, it is also important to know the network or behavioral relevance of these effects. Because extrapolations between different levels in the nervous system must be made with caution, it is necessary to examine effects directly at each of these levels. Spinal reflexes were used to investigate the behavioral effects of sensory modulation in mammals (see Wiesenfeld-Hallin 1995). However, in these preparations, it is difficult to obtain detailed mechanistic explanations at the cellular and synaptic levels. Conversely, although detailed cellular information was obtained with dissociated cells or tissue slices (see Murase et al. 1989), the network or behavioral relevance of these results must be extrapolated.

In the lamprey, a lower vertebrate, the effects of neuromodulators on sensory circuitry can be examined on identified neurons and monosynaptic connections in the intact spinal cord (see Christensen and Grillner 1991; El Manira et al. 1997; Parker and Grillner 1996). These neurons include the cutaneous touch and pressure-sensitive dorsal cells, which have large intraspinal cell bodies, and large spinobulbar neurons, which receive monosynaptic glutamatergic inputs from dorsal cells and other primary afferents (Brodin et al. 1987; Rovainen 1967). We examined the relevance of the modulation of sensory circuitry with reflex responses evoked by electrical stimulation of the attached tail fin in an isolated spinal cord preparation (McClellan and Grillner 1983). Reflex responses can be monitored by recording extracellularly from spinal ventral roots the activity corresponding to that recorded myographically in intact animals (McClellan and Grillner 1983). In addition, the effects of neuromodulators on sensory inputs can be examined independently of their direct effects on motor or premotor neurons (see Parker and Grillner 1998) by dividing the spinal cord into separate pools, thus allowing the relevance of sensory modulation to be determined unequivocally.

The spinal dorsal horn contains a large number of neuropeptides (see Nyberg et al. 1995), although in most cases their effects are unclear (see DISCUSSION). Many of these peptide systems were conserved throughout vertebrate evolution (see Brodin et al. 1995), and thus the lamprey is a relevant model system in which to examine neuropeptide-mediated sensory modulation. Tachykinin immunoreactivity in the lamprey is found in the dorsal root, dorsal column, and dorsal horn (Van Dongen et al. 1986) and in close apposition to sensory dorsal column axons (Svensson and Grillner, unpublished observations). Tachykinins have excitatory effects on the sensory circuitry in that they depolarize mechanosensory dorsal cells, increase the excitability of dorsal cells and second-order spinobulbar neurons, and presynaptically potentiate excitatory but reduce inhibitory, synaptic inputs (Parker and Grillner 1996). Certain of these effects are mediated through protein kinase C (Parker et al. 1997). Neuropeptide Y (NPY) immunoreactivity is found in small bipolar interneurons, the axons of which are
found in close apposition to axons in the dorsal column and dorsal horn (Bongianni et al. 1990). NPY has the opposite effect of tachykinins in that it reduces the excitability of spinobulbar interneurons and presynaptically reduces dorsal cell-mediated glutamatergic synaptic transmission (Parker et al. 1998b). GABA is colocalized with NPY in bipolar neurons in the dorsal column (Parker et al. 1998b). The GABA_B receptor agonist baclofen has complementary effects to NPY in depressing sensory synaptic transmission (Christenson et al. 1991; Parker et al. 1998b).

Because the effects of neuropeptides were characterized at the cellular and synaptic levels, the aim of this study was to examine their actions at the network level by investigating their effects on spinal reflexes. In addition, we examined the effects of the neuropeptide bombesin, which is colocalized with 5-hydroxytryptamine (5-HT) and calcitonin gene-related peptide (CGRP) in primary afferents (Brodin et al. 1988) at the cellular, synaptic, and reflex levels.

METHODS

Adult male and female lampreys (*Lampetra fluviatilis*) were anaesthetised with tricaine methane sulphonate (MS-222; Sandoz, Switzerland), and the spinal cord and notochord were dissected from the mid-trunk region to the tail fin (~50 segments). The tail fin was left attached, and the preparation was placed dorsal side up in a Sylgard (Dow Corning)-lined chamber and superfused with Ringer containing (in mM) 119.8 NaCl, 2.1 KCl, 0.1 CaCl₂, 7.2 MgCl₂, 4 glucose, 2 HEPES, and 0.5 L-glutamine. Low calcium–high magnesium Ringer contained (in mM) 138 NaCl, 2.1 KCl, 1.8 CaCl₂, 1.2 MgCl₂, 4 glucose, 2 HEPES, and 0.5 L-glutamine. All solutions were bubbled with O₂, and the pH was adjusted to 7.4 with 1 M NaOH. The experimental chamber was kept at a temperature of 10–12°C.

The connective tissue and meninx primitiva were removed from the dorsal surface of the spinal cord. The spinal cord–notochord preparation was split into three pools by placing Vaseline barriers at different positions (see Fig. 1A). One barrier was placed ~15 segments rostral to the tip of the tail fin, with a second barrier placed ~20 segments more rostral to this barrier. High divalent cation or low calcium/high magnesium Ringer was placed in the middle pool to block polysynaptic transmission through this pool. Thus the input to the rostral pool is due to axons from the caudal pool that project beyond the middle pool. Extra-cellular ventral root and intracellular recordings are made from the rostral pool. Graph of the ventral root burst area (Bi) and traces showing raw and rectified and integrated ventral root bursts (Bii) showing that high divalent cation Ringer reduces ventral root activity recorded in the rostral pool, presumably because of the reduction of polysynaptic inputs. The bar underneath the trace indicates the onset and duration of the tail fin stimulation (see METHODS).

![Diagram of the isolated spinal cord-tail fin preparation. The spinal cord is separated by Vaseline barriers into a tail fin pool (~15 segments), a middle pool (~20 segments), and a rostral pool. The tail fin is stimulated electrically by square silver electrodes placed on either side of the tail. Neur modulators are added to the tail fin bath. High divalent cation or low calcium/high magnesium Ringer is placed in the middle pool to block polysynaptic transmission through this pool. Thus the input to the rostral pool is due to axons from the caudal pool that project beyond the middle pool. Extra-cellular ventral root and intracellular recordings are made from the rostral pool. Graph of the ventral root burst area (Bi) and traces showing raw and rectified and integrated ventral root bursts (Bii) showing that high divalent cation Ringer reduces ventral root activity recorded in the rostral pool, presumably because of the reduction of polysynaptic inputs. The bar underneath the trace indicates the onset and duration of the tail fin stimulation (see METHODS).](http://jn.physiology.org/)

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**FIG. 1.** A: diagram of the isolated spinal cord-tail fin preparation. The spinal cord is separated by Vaseline barriers into a tail fin pool (~15 segments), a middle pool (~20 segments), and a rostral pool. The tail fin is stimulated electrically by square silver electrodes placed on either side of the tail. Neur modulators are added to the tail fin bath. High divalent cation or low calcium/high magnesium Ringer is placed in the middle pool to block polysynaptic transmission through this pool. Thus the input to the rostral pool is due to axons from the caudal pool that project beyond the middle pool. Extra-cellular ventral root and intracellular recordings are made from the rostral pool. Graph of the ventral root burst area (Bi) and traces showing raw and rectified and integrated ventral root bursts (Bii) showing that high divalent cation Ringer reduces ventral root activity recorded in the rostral pool, presumably because of the reduction of polysynaptic inputs. The bar underneath the trace indicates the onset and duration of the tail fin stimulation (see METHODS).
side changed during the experiment \((n = 2)\) were not included in the analysis.

Extracellular recordings were made from ventral roots in the rostral pool with glass suction electrodes. Intracellular recordings were also made from motor neurons or unidentified gray matter neurons in the rostral pool with thin-walled glass micropipettes filled with 4 M K acetate and with resistances of 40–60 MΩ. To facilitate intracellular recordings, in some experiments approximately three segments of the rostral end of the spinal cord were isolated from the notochord. This region of the spinal cord was stabilized by placing a plastic net over it, which was secured by pinning it onto the Sylgard. Care was taken not to stretch the cord where it was still attached to the notochord. Motor neurons were identified by recording 1:1 orthodromic spikes in the adjacent ventral root after current injection into the soma. An Axoclamp 2A amplifier was used for amplification and in discontinuous current-clamp mode for current injection.

To quantify the effects of neuromodulators on ventral root responses, ventral root activity was analyzed off-line with pClamp software (version 6, Axon Instruments). The burst was first rectified and then integrated over time (see Fig. 1Bii). The area of the burst (i.e., the train of spikes evoked by tail fin stimulation) was measured as the peak of the integrated trace. The area was measured for as long as the burst evoked by tail fin stimulation occurred, typically <500 ms. Three trials were performed in control, in the presence of the drug, and after wash-off. Each trial was separated by a 2-min interval. This delay between trials ensured that the stimulation trials themselves did not cause any changes in the intensity of the ventral root response (data not shown). The effect of tail fin stimulation on ongoing locomotor activity and its modulation was examined by eliciting fictive locomotion in the rostral most pool by applying N-methyl-D-aspartate (NMDA; 150 μM) (Brodin et al. 1985). In this case, the frequency was measured in 2-s bins, before and after substance P application.

To investigate the cellular and synaptic effects of bombesin, a piece of the caudal region of the spinal cord (≈10 segments) was isolated from the notochord. Action potentials were elicited in dorsal cells and spinobulbar neurons by the injection of 1-ms depolarizing current pulses of 10–20 nA. Four action potentials were elicited at 1 Hz in control and then in the presence of bombesin. Stimulation at this frequency did not cause any action-dependent changes in the action potential (data not shown). The spikes in each trial were averaged for analysis. The spike amplitude was measured from the baseline preceding the spike to the peak of the spike, and the AHP amplitude was measured from the baseline preceding the spike to the maximum hyperpolarized potential reached after the spike. The spike duration was measured at one-half height. Excitability and input resistance were examined by injecting 100-ms depolarizing or hyperpolarizing current pulses, respectively, of 1–5 nA into the dorsal cell or spinobulbar neuron somata. Dorsal cells and spinobulbar neurons were identified by their characteristic shapes and positions in the spinal cord (Rovainen 1967). Synaptic inputs to spinobulbar neurons were evoked by stimulating the dorsal column caudal to the spinobulbar neuron in an isolated piece of spinal cord (≈10 segments). High divalent cation Ringer was used to block polysynaptic inputs when stimulating the dorsal column. The lateral tracts and gray matter were lesioned rostral to the stimulating electrode to prevent inputs to spinobulbar interneurons from neurons in these areas. Axon Instruments software (Axotape and pClamp) was used for data acquisition, and analysis was performed on a 486 PC computer equipped with an A/D interface (Digitdata 1200, Axon Instruments, CA).

Substance P acetate, bombesin acetate, and porcine NPY were obtained from Sigma. Baclofen was obtained from Tocris (Bristol, UK). Unless stated otherwise, statistical significance was examined with two-tailed, paired \(t\)-tests. The statistical values refer to the pooled data from the number of experiments indicated in each case. Results are expressed as means ± SE; \(n\) numbers given in the text refer to the number of animals studied.

**RESULTS**

**Effects of substance P on reflex responses**

The tachykinin substance P depolarizes mechanosensory dorsal cells, increases the excitability of dorsal cells and spinobulbar neurons, and increases dorsal root and dorsal column-evoked glutamatergic inputs but reduces dorsal column-evoked inhibitory inputs (Parker and Grillner 1996). The behavioral effect of this sensory modulation was examined by stimulating the tail fin in the isolated spinal cord–tail fin preparation (see Fig. 2A, inset). Application of substance P (100 nM) to the tail fin pool increased the intensity of the tail fin stimulation-evoked ventral root activity in the rostral pool (Fig. 2, A and B; \(n = 8/10\); \(P < 0.05\)). In the remaining two preparations, substance P had no effect on the ventral root response. Substance P also increased the tail fin stimulation-evoked synaptic response in motor neurons \((n = 3)\) and unidentified gray matter neurons \((n = 1)\) in the rostral pool (Fig. 2D; \(P < 0.01\)). In cells that spiked in control, the number of spikes evoked by the stimulation was increased \((n = 3); \)see Fig. 2A). These effects of substance P partly recovered after washing for 1 h (Fig. 2, B–D).

Substance P has a biphasic concentration-dependent effect on sensory neurons, the maximal effect occurring with 100 nM substance P, but a reduced or even opposite effect occurring with 1 μM (Parker et al. 1997). The modulation of tail fin-evoked responses also exhibited this concentration-dependent trend (Fig. 2C); 100 nM substance P usually increased tail fin stimulation-evoked ventral root responses \((n = 8/10); \) \(P < 0.05\), whereas 1 μM substance P had a smaller \((n = 3)\) or even opposite \((n = 2)\) effect.

The tachykinin antagonist Spantide II \((4 \mu M)\) (Parker and Grillner 1996) blocked the facilitating effect of 100 nM substance P on tail fin stimulation-evoked ventral root responses (Fig. 2E; \(n = 4/4\); \(P > 0.1\)), suggesting that the effect was mediated through tachykinin receptors. A second application of substance P after washing off Spantide II for ≥1 h could result in an increase in the ventral root response \((n = 2/4); \) Fig. 2E).

**Effects of substance P on tail fin stimulation-evoked modulation of locomotor activity**

Locomotor activity can be elicited in the lamprey spinal cord by bath application of excitatory amino acids (Cohen and Wallén 1980; Poon 1980). To examine the effects of sensory input and its modulation on ongoing locomotor activity, NMDA was applied to the rostral pool of the isolated spinal cord–tail fin preparation (Fig. 3Aii). Stimulating the tail fin increased the frequency of rostral ventral root bursts (Fig. 3; \(n = 4/4\); \(P < 0.05\)). The addition of substance P \((100 \text{ nM})\) to the tail fin pool had two effects. First, it increased the basal frequency of NMDA-evoked ventral root responses, presumably because of the tonic activation of ascending neurons (Parker and Grillner 1998). In addition, substance P facilitated the increase in burst frequency evoked by tail fin stimulation (Fig. 3; \(n = 4/4\); \(P < 0.05\)).

**Effects of NPY and baclofen on reflex responses**

NPY immunoreactivity is found in the lamprey dorsal column and dorsal horn (Parker et al. 1998b) and in close appo-
sition to dorsal column axons, including the axons of mechanosensory dorsal cells (Bongianni et al. 1990). NPY presynaptically reduces glutamatergic inputs from the dorsal cells and reduces the excitability of spinobulbar neurons (Parker et al. 1998b). Application of NPY (100 nM) (Parker et al. 1998b) to the tail fin pool of the isolated spinal cord-tail fin preparation reduced the ventral root activity recorded in the rostral pool (Fig. 4, A and B; n = 6/7; P < 0.05). This effect was associated with a reduction in the peak amplitude of ascending synaptic inputs to motor neurons (n = 2) and unidentified gray matter neurons (n = 1) in the rostral pool (Fig. 4C; P < 0.05) or with a reduction in the number of spikes evoked in neurons that spiked in control (n = 2; Fig. 4A). The effects of NPY on ventral root activity were greater when the initial activity level was high. For example, after potentiating reflex activity with 100 nM substance P, NPY reduced the response by 61.5 ± 11.2 compared with 35.3 ± 9 when it was applied to a control cord (n = 3; Fig. 4D). These effects of NPY recovered partially after washing for 1 h (Fig. 4, B and C).

FIG. 2. The effects of substance P (100 nM) on ascending inputs and reflex activity. A: activity recorded extracellularly from a ventral root and intracellularly from a motor neuron in the rostral pool. Substance P increased the intracellular response in the motor neuron and potentiated the ventral root activity. The spikes are clipped in this and subsequent figures. Inset in this and subsequent figures shows a schematic diagram of the experimental preparation. B: graph showing data from a single experiment in which the 3 trials in control, in the presence of 100 nM substance P, and after wash-off are shown. C: graph showing the effect of 100 nM and 1 μM substance P on the area of the ventral root burst compared with control. Data from 10 experiments is shown with 100 nM and 5 experiments with 1 μM. D: graph with data from 4 cells in which spikes were not elicited by tail fin stimulation, showing the effect of substance P (100 nM) on the peak amplitude of the tail fin stimulation-evoked depolarization. E: effects of substance P (100 nM) on the area of the ventral root burst are blocked by the tachykinin antagonist spantide II (4 μM). Substance P could result in an increase in ventral root area when spantide II was washed off. Data from 2 experiments are shown in E.
As in higher vertebrates (see Todd and Spike 1993), NPY colocalizes with GABA in bipolar cells in the dorsal column (Parker et al. 1998b). The GABA<sub>P</sub> receptor agonist baclofen has complementary but not identical effects to NPY in presynaptically modulating glutamatergic synaptic transmission from sensory neurons to spinobulbar neurons (Parker et al. 1998b). The effect of baclofen (10 μM) (Parker et al. 1998b) on reflex responses was thus also investigated. Baclofen reduced the ventral root activity in the rostral pool (Fig. 5, A and B; n = 4/4; P < 0.01) and also reduced the peak amplitude of ascending synaptic inputs or spiking in motor neurons (n = 4/4; Fig. 5, A and C). These effects of baclofen largely recovered after washing for 1 h (Fig. 5, B and C).

Although baclofen and NPY have qualitatively similar effects on glutamatergic synaptic transmission to spinobulbar neurons, their effects are quantitatively different in that baclofen has a larger maximal effect that recovers faster than the effect of NPY (Parker et al. 1998b). A similar quantitative difference was seen in the effects of baclofen and NPY on tail fin-evoked responses. Baclofen resulted in a larger reduction of the ventral root burst area (71 ± 4.6) than NPY (35.3 ± 9; P < 0.05, two-tailed, independent t-test). In addition, the extent of the recovery after washing for 1 h was greater for baclofen than for NPY (baclofen 43.1 ± 4; NPY 16 ± 10.3; compare Figs. 4, B and C, and 5, B and C).

**Effects of bombesin on cellular, synaptic, and reflex properties**

The previous experiments examined the reflex effects of neuromodulators previously studied with regard to cellular and synaptic sensory modulation. In addition, we examined the effects of the neuropeptide bombesin, which was not previously examined at any level in the lamprey. Bombesin colocalizes with 5-HT and CGRP in afferent axons (Brodin et al. 1988). At the reflex level bombesin (100 nM) mimicked the effects of substance P in that it increased the ventral root activity in the rostral pool (Fig. 6, A and B; n = 5/6; P < 0.01) and increased synaptic input and spiking in motor neurons (Fig. 6, A and C; n = 3) and unidentified gray matter neurons (n = 1; P < 0.05). However, in contrast to the effects of substance P, no appreciable recovery of the effects of bombesin were seen after washing for 2 h (Fig. 6C).

As with substance P (Parker and Grillner 1996), bombesin (100–500 nM) did not significantly modulate the mechanosensory dorsal cell input resistance (data not shown), and in contrast to substance P bombesin did not affect the action potential properties of any dorsal cell examined (Fig. 7, A and B; n = 11). Bombesin (100 nM) had variable effects on the excitability of mechanosensory neurons, an increase being seen in 5 of 11 dorsal cells. However, a consistent effect on excitability was seen when the dorsal cells were divided into those that responded to a 100–ms depolarizing current pulse with a train of spikes and those that responded with only one or two spikes, putatively slowly adapting pressure and rapidly adapting touch-sensitive dorsal cells, respectively (Christenson et al. 1988). Bombesin had an effect on the excitability of only one of six touch-sensitive cells (Fig. 7C) but increased the excitability in four of five pressure-sensitive cells (Fig. 7D). Increasing the bombesin concentration to 500 nM made the effect on the excitability of putative pressure-sensitive cells less consistent, an increase occurring in two of five cells. The excitability of putative touch-sensitive neurons was again not affected by higher bombesin concentrations (n = 6; data not shown).

Finally, the synaptic effects of bombesin were examined by evoking depolarizing synaptic potentials in spinobulbar interneurons by extracellular stimulation of the dorsal column, activating the axons of dorsal cells and other sensory axons. Bombesin (100 nM) increased the amplitude of the synaptic potential in spinobulbar neurons (n = 4/5; P < 0.05) independently of an effect on the input resistance of membrane potential of the spinobulbar neuron. These effects were also long lasting, recovery occurring after washing in excess of 2 h (Fig. 8, Ai and Aii).

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**FIG. 3.** The effects of substance P on tail fin stimulation-evoked modulation of locomotor activity. The tail fin was stimulated at the start of a ventral root burst. This resulted in an increase in the frequency of ventral root activity elicited by N-methyl-D-aspartate (150 μM) in the rostral pool (Ai). Each bar represents the frequency in 2-s time bins. Substance P increased the frequency of ventral root bursts in the absence of tail fin stimulation and potentiated the effects of tail fin stimulation on burst frequency. Data from four experiments is shown. Aii: traces showing the effects of tail fin stimulation on ventral root activity in control and in the presence of substance P. The bar under the trace indicates the onset and duration of tail fin stimulation.
DISCUSSION

The possible network relevance of neuropeptide-mediated sensory modulation, as monitored by reflex responses after cutaneous stimulation, was examined in this study. The results suggest facilitatory and inhibitory roles, respectively, for substance P and NPY/baclofen in modulating cutaneous stimulation-evoked responses, in agreement with previous studies at the cellular and synaptic levels (Parker and Grillner 1996; Parker et al. 1998b). We have also shown that bombesin potentiates reflex activity and have examined the cellular and synaptic mechanisms underlying this effect.

Lampetra NPY was sequenced and has strong homology to mammalian NPY (Söderberg et al. 1994). In a previous electrophysiological study (Parker et al. 1998b) no significant difference in the cellular and synaptic effects of synthetic Lampetra NPY, porcine NPY, and mammalian NPY were found, suggesting that this peptide is also functionally conserved. Lampetra tachykinins were also sequenced, the functionally important C terminal having strong homology to higher vertebrates (Waugh et al. 1995). Molluscan, amphibian, and mammalian tachykinins also had quantitatively similar effects at the sensory and network levels (Parker and Grillner 1996; Parker et al. 1998b). We have also shown that bombesin potentiates reflex activity and have examined the cellular and synaptic mechanisms underlying this effect.

FIG. 4. Effects of neuropeptide Y (NPY: 100 nM) on ascending inputs and reflex activity. A: activity recorded extracellularly from a ventral root and intracellularly from a motor neuron in the rostral pool. B: graph showing the effect of NPY on the ventral root burst area. Data from 7 experiments are shown. C: graph with data from 3 cells that did not spike in response to tail fin stimulation, showing the effect of NPY on the amplitude of the tail fin stimulation-evoked depolarization. D: graph showing the increased effect of NPY (100 nM) on the ventral root burst area after it was first potentiated by substance P (100 nM). Data from 3 experiments are shown.
1996; Parker et al. 1998a), again suggesting functional conservation of this peptide. Although the structure of Lampetra bombesin is currently unknown and thus it is possible that the commercial peptide used here does not fully match the effects of endogenous bombesin, the conservation of the functional effects of NPY and tachykinins suggests that the effects of exogenous bombesin shown here will resemble those of the endogenous peptide.

The preparation used (see Fig. 1) was designed to allow the effects of neuromodulators to be examined on the sensory level in the absence of modulator-induced effects on motor and premotor interneurons. Drugs were applied to the caudal-most region of the spinal cord, where the axons of sensory neurons activated by tail fin stimulation enter the spinal cord. Low calcium/high magnesium Ringer or high divalent cation Ringer was applied to the middle pool to reduce synaptic transmission or raise the threshold for activation of neurons in the middle pool (Berry and Pentreath 1976). This will prevent rostral pool neurons from being activated through a chain of ascending local interactions, thus limiting the stimulation-induced input to the rostral pool to caudal pool neurons with ascending axons that project directly to the rostral pool. This will include dorsal root and dorsal column axons (Dubuc et al. 1992), including those of mechanosensory dorsal cells and spinobulbar neurons (Rovainen 1967, 1974) and propriospinal neurons with long ascending axons in the lateral column (Vinay et al. 1998). Dorsal cell, dorsal root, and dorsal column axons make monosynaptic connections with spinobulbar neurons and polysynaptic excitatory and inhibitory connections to motor and premotor neurons (Birnberger and Rovainen 1971; Rovainen 1967). Although intracellular stimulation of single spinobulbar neurons was not sufficient to elicit reflex responses (Teräväinen and Rovainen 1971), the combined activation of sensory dorsal column axons and spinobulbar neurons appears to mediate tail fin stimulation-evoked reflex responses (McClellan and Grillner 1983). Thus the neurons examined in the previous cellular and synaptic studies contribute to the input to motor and premotor neurons in the rostral pool and thus influence the ventral root activity elicited in response to tail fin stimulation.

**Comparison of the effects of substance P, NPY, and baclofen at the cellular/synaptic and reflex levels**

Substance P has excitatory effects on sensory dorsal cells and spinobulbar neurons and potentiates dorsal column and dorsal root-evoked synaptic inputs to spinobulbar neurons (Parker and Grillner 1996). In the experiments presented here, substance P was shown to potentiate the effects of ascending synaptic inputs and increase ventral root activity in the rostral pool. In addition, the concentration dependence of the effects on ventral root responses in this study matched the concentration dependence previously found at the cellular and synaptic level.

![Fig. 5](http://jn.physiology.org/). The effects of baclofen (10 μM) on ascending inputs and reflex activity. A: activity recorded extracellularly from a ventral root and intracellularly from a motor neuron in the rostral pool. B: graph showing the effect of baclofen on the area of the ventral root burst. Data from 4 experiments are shown. C: graph with data from 2 cells in which spikes were not elicited by tail fin stimulation, showing the effect of baclofen on the amplitude of the tail fin stimulation-evoked depolarization and the recovery after washing for 1 h.
levels (Parker et al. 1997), maximal effects being obtained with 100 nM substance P, but reduced or opposite effects being seen with higher concentrations.

NPY has opposite cellular and synaptic effects to substance P in that it reduces the excitability of spinobulbar neurons and presynaptically depresses dorsal cell, dorsal root, and dorsal column-evoked inputs to spinobulbar neurons (Parker et al. 1998b). NPY colocalizes with GABA in bipolar neurons in the dorsal column (Parker et al. 1998b). The GABAB receptor agonist baclofen qualitatively mimics the effects of NPY in presynaptically reducing glutamatergic afferent-evoked inputs to spinobulbar neurons. However, baclofen and NPY have quantitatively different effects at the synaptic level in that baclofen has a larger effect that recovers more rapidly than that of NPY. In this paper, both NPY and baclofen reduced ascending inputs to rostral locomotor network neurons and reduced rostral ventral root activity. Quantitative differences between the effects of NPY and baclofen were also seen, baclofen again having a larger effect than NPY, which recovers to a greater extent after washing for 1 h. The modulation of reflex activity is thus consistent with the effects of substance P, NPY, and baclofen at the cellular and synaptic levels.

Role of bombesin in modulating sensory input

Bombesin (100 nM) increased tail fin stimulation-evoked ascending inputs and ventral root activity, thus mimicking the effects of substance P. The cellular and synaptic effects of bombesin in the lamprey were not previously examined, but the effects on ascending inputs and reflex responses suggested that bombesin would have excitatory effects at the cellular and synaptic levels. As with substance P, bombesin increased the excitability of the dorsal cells. Unlike substance P, however, this effect was only seen on putative pressure-sensitive dorsal cells, i.e., those that responded to a depolarizing current pulse with a train of spikes (Christenson et al. 1988). Bombesin also potentiated dorsal column stimulation-evoked inputs to spinobulbar neurons, thus mimicking the effects of substance P on sensory synaptic transmission. Unlike substance P, however, bombesin did not affect the membrane potential or action potential properties of the dorsal cells. Because bombesin did not affect the resting input resistance or the properties of dorsal cell action potentials, it may increase the excitability of the dorsal cells by acting on voltage-activated conductances that do not contribute significantly to the depolarizing or repolarizing phases of the action potential. The effect of bombesin on the excitability of putative pressure-sensitive dorsal cells appears to be concentration dependent because, in contrast to 100 nM, 500 nM bombesin had no consistent effect on the excitability of pressure-sensitive dorsal cells (data not shown).

Bombesin facilitates the tail flick reflex in the rat (Cridland and Henry 1992), and the bombesin agonist neuromedin C enhances mechanical nociception in the rat paw pressure test (Onogi et al. 1995). However, bombesin and
neuromedin B and C reduced spontaneous and evoked synaptic inputs to superficial dorsal horn neurons (De Koninck and Henry 1989), an effect that is not consistent with its effects on reflex activity. The concentration-dependent effect of bombesin, shown in this study, could contribute to the inconsistent effects of bombesin at the reflex and cellular levels (Cridland and Henry 1992; De Koninck and Henry 1989) because the cellular study used ionophoresis of 1 mM bombesin, and the latter study used intrathecal administration of low nM concentrations. The concentration-dependent effects of bombesin at the reflex and synaptic levels in the lamprey remain to be investigated.

Role of the sensory modulation

Substance P and bombesin are found in separate populations of primary afferents (Brodin et al. 1988; Van Dongen et al. 1986), whereas NPY and GABA are colocalized in bipolar interneurons in the dorsal column (Parker et al. 1998b). The results of this and previous studies (Parker and Grillner 1996; Parker et al. 1997) suggest that activation of substance P and bombesin-containing afferents will facilitate sensory inputs and thus sensory-evoked responses through cellular and synaptic effects on other primary afferents and sensory interneurons. Conversely, activation of NPY/GABAergic interneurons will inhibit sensory-evoked responses through presynaptic inhibition of afferent synaptic trans-

FIG. 7. The effects of bombesin (100 nM) on the cellular properties of the mechanosensory dorsal cells. A: overlaid averaged (n = 4) dorsal cell action potentials in control and after application of bombesin, showing the lack of effect of bombesin. B: graphs showing the lack of effect of bombesin on dorsal cell action potential properties (n = 11). C: bombesin did not affect the excitability of dorsal cells that spiked once in response to a 100-ms depolarizing current pulse (rapidly adapting or touch-sensitive cells; n = 6) but did increase the excitability of neurons that spiked several times in response to the current pulse (slowly adapting or pressure-sensitive cells; n = 5). D: insets in C and D show responses to dorsal cells of the 2 types in response to a 100-ms depolarizing current pulse in control (top) and after the application of 100 nM bombesin for 10 min (bottom).

FIG. 8. Bombesin potentiates afferent synaptic transmission. Ai: effects of bombesin (100 nM) on the excitatory postsynaptic potential (EPSP) elicited in spinobulbar neurons by stimulation of the dorsal column. The bar indicates the onset and duration of bombesin application. Aii: traces showing averaged EPSPs (n = 20) in control and in the presence of bombesin.
mission and a reduction in the excitability of sensory interneurons (Parker et al. 1998b). 5-HT also presynaptically inhibits afferent inputs (El Manira et al. 1997) and colocalizes with bombesin and CGRP in primary afferents (Brodin et al. 1988). These afferents would thus be able to facilitate or inhibit sensory inputs if a mechanism exists for the selective release of bombesin or 5-HT. Sensory input to the spinal cord is thus subject to presynaptic and/or postsynaptic facilitation or inhibition by different spinal or afferent systems.

**CONCLUSION**

These results and those of previous papers (Christensen and Grillner 1991; El Manira et al. 1997; Parker and Grillner 1996; Parker et al. 1998b) show that the modulation of sensory inputs can be examined directly at the behavioral, cellular, and synaptic levels in the intact lamprey spinal cord. In mammalian preparations, the roles of many neuropeptides are unclear, with sometimes contradictory effects being reported. For example, bombesin depresses sensory synaptic inputs to nociceptive neurons in the superficial dorsal horn (De Koninck and Henry 1989) but induces or facilitates nociceptive reflex responses (Cridland and Henry 1992; Mao et al. 1992). Somatostatin inhibits responses of dorsal horn neurons to thermal stimulation (Sandkühler et al. 1990) but facilitates thermal stimulation-evoked reflexes (Wiesenfeld-Hallin 1986). In addition, whereas somatostatin inhibits nociceptive inputs (Randic and Miletic 1978), it facilitates nociceptive behaviors, including the hindlimb flexion reflex and scratching (Wiesenfeld-Hallin 1985). In this study, it was possible to separate the sensory effects of neuromodulators from those exerted on premotor interneurons and motor neurons. This is of crucial importance. For example, in addition to its sensory effects (Parker and Grillner 1996), substance P also has specific effects on all components of the locomotor network (motor neurons and premotor interneurons) (Parker and Grillner 1998). Effects of intrathecally administered modulators at the motor or premotor level could thus contribute to the variability in the reported effects in mammalian preparations and the lack of consistency between results at the cellular and network levels, thus illustrating the difficulties involved in extrapolating functional effects among different levels in the nervous system.

Because the types and spinal locations of the neuropeptides examined here correspond to those in higher vertebrates (Brodin and Grillner 1990; Brodin et al. 1995), these results are presumably also relevant to other vertebrate systems. Given the possibility to examine the effects of these modulators in the intact spinal cord at the cellular, synaptic, and reflex levels, the lamprey provides a useful model system in which to examine the role and underlying mechanisms of neuromodulators in modulating sensory inputs.

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