Effects of Serotonin and Nitric Oxide on Mid-gut Motility in Tobacco Hornworm (*Manduca sexta*) Larvae

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ABSTRACT

The anterior mid-gut of the tobacco hornworm, *Manduca sexta*, is active in mixing freshly ingested diet with gut secretion. Spontaneous muscle activity is lost when the anterior gut is isolated, suggesting that neural and/or humoral inputs may be important in sustaining normal activity in vivo. In preliminary studies, both serotonin and cGMP (the putative second messenger for NO) were visualized in the anterior mid-gut by immunohistochemistry. We have also shown that serotonin and sodium nitroprusside (SNP), a NO donor, have measurable effects on the contractile state of isolated anterior gut. In these studies both serotonin and SNP caused a long-lasting contraction of longitudinal and circular muscle mounts.

INTRODUCTION

The anterior mid-gut of the tobacco hornworm *Manduca sexta* is active in mixing freshly ingested diet with gut secretion. The gut muscularis consists of a diffuse layer of circular fibers and six discrete bands of longitudinal fibers that run the length of the gut (Nardi, 1990). The gut is innervated by the central nervous system by way of the recurrent nerve that passes from the frontal ganglion along the dorsal foregut (Miles and Booker, 1994). This pathway branches at the junction of foregut and mid-gut to enter the enteric plexus, which contains intrinsic neurons. The roles of central inputs and intrinsic neurons in generating patterned gut activity have not been clarified. However, the general picture suggests that patterned output that originates in the frontal ganglion drives coordinated movements of the fore- and mid-gut as shown in Figure 1 (Copenhaver and Taghert, 1991).

Nitric oxide synthase produces nitric oxide in central nervous system neuron. Nitric oxide (NO) is part of a signaling molecule which leads to the formation of cyclic GMP (cGMP) in target cells (Bicker, 2001). Cyclic GMP causes the gut neuron to become more active, increasing release of serotonin (5-HT) onto the muscle, exciting it (Muller and Hildebrandt, 1995).

In these studies, serotonin, nitric oxide synthase (a marker for nitridergic neurotransmission) and cGMP (the putative second messenger for NO) have been visualized by immunohistochemistry in neurons innervating the anterior mid-gut. Changes in activity of longitudinal and circular muscle in response to serotonin and sodium nitroprusside (SNP, a NO donor) were measured *in vitro*. 
RESEARCH OBJECTIVES

1. To show the distribution of serotonergic and nitroxidergic neurons innervating the gut.
2. To measure effects of serotonin and nitric oxide on muscular force development.

Figure 1. The fore- and mid-gut of the tobacco hornworm Manduca Sexta.

METHODS

Immunohistochemistry of serotonin, nitric oxide synthase and cGMP was conducted. Freshly dissected gut tissue was fixed in 2% paraformaldehyde. Whole-mounts were treated with one or more primary antibodies to serotonin, universal nitric oxide synthase and cGMP. The primary antibodies were localized with secondary antibodies conjugated with rhodamine red (red), fluorescein isothiocyanate (yellow) or AMCA (blue).

To determine the effects of serotonin and NO on mechanical activity, gut tissue from 5th instar tobacco hornworms (Manduca sexta) was obtained by dissection of cold-anesthetized larvae weighing 6-13 gm. For studies of circular muscle (Figure 2), approximately 1 mm wide rings of gut were mounted horizontally between two hooks. One hook was fixed and the other was attached to an isometric strain gauge (UFI model 1030). For studies of the activity of longitudinal muscle, vertically-suspended gut segments 5-7 mm long were fastened to a rigid bar at one end and to the strain gauge at the other (see Figure 2). In both cases, the muscle was bathed in O₂-saturated lepidopteran saline at room temperature. Digitized data were recorded and analyzed using Sable Systems software. Serotonin (5-HT) and sodium nitroprusside (SNP) were added from concentrated aqueous stock solutions to final concentrations as indicated.
RESULTS

Immunochemistry. NOS and serotonin axons run along the length of the gut on each side of the bands of longitudinal muscle. Figure 3 shows serotonergic axons with axon varicosities suggestive of synaptic contacts with the muscle, running on each side of a longitudinal muscle band. The nitridergic axons do not show similar varicosities.

Figure 4 shows isoviews of the same tissue, which was triple-stained for NOS, serotonin and cGMP. The close association of the nitridergic axons with the serotonin-positive cell body and the fact that the serotonin-positive cell body is positive for cGMP suggests that the nitridergic axons, possibly emanating from cell bodies in the frontal ganglion, synapse on serotonin neurons in the gut, which then appear to synapse on the longitudinal muscle fibers.

Figure 2. Muscle activity studies.

Muscle Dynamics. Further experiments showed that both SNP and serotonin caused an initial relaxation of longitudinal muscle lasting 1-2 min, followed by a longer contraction lasting up to 20 min. (see Figure 5). SNP and serotonin on circular muscle showed, as with longitudinal muscle, that both SNP and serotonin caused long-duration contractions of circular muscle rings (see Figure 5 and Figure 6).

Figure 3. Serotonergic axons with axon varicosities on each side of a longitudinal muscle band.
Figure 4. Isoviews of a longitudinal muscle band triple-stained with NOS (a), Serotonin (b), and cGMP (c).

a. 

b. 

c. 

Figure 5. Longitudinal muscle contractions.  Figure 6. Circular muscle contractions.

DISCUSSION

The isolated gut of Manduca sexta larvae is largely quiescent when placed in the muscle chamber, and does not show the spontaneous, cyclic activity typical of many preparations of visceral muscle. This finding indicates that exogenous motor input is paramount in generating
mixing and peristaltic contractions of the gut observable in vivo. The gross anatomy of potential motor pathways has been studied (Muller and Hildebrandt, 1995), but there is relatively little information about the nature and specific effects of neurochemical transmitters in mid-gut muscle of lepidoperan larvae.

These studies showed that the gut is innervated by neurons immunoreactive for serotonin and cGMP and suggest that the nitridergic neurons are postsynaptic to the serotonin neurons. The importance of such pathways for motor control of the gut is supported by the finding that both exogenous serotonin and NO donor elicit similar increases in muscle tone. The long timescale of the responses to serotonin and NO was unexpected, but is consistent with a modulatory style of action. This suggests that the neurotransmitters that drive short timescale responses remain to be elucidated. This suggestion is supported by our finding that the number of neurons detectable with methylene blue staining is considerably in excess of the number that are immunoreactive to serotonin or cGMP.

CONCLUSION

Immunohistochemistry confirmed that the gut is innervated by nitridergic and serotonergic neurons. This suggested that the serotonergic neurons may be postsynaptic to the nitridergic neurons.

In the muscle dynamics experiments, both SNP and serotonin caused long-lasting contractions of both longitudinal and circular muscle. This is consistent with an excitatory effect of NO on serotonin neurons.

The relative lack of patterned activity when the gut is mounted as a tube suggests that much or all of the patterned activity seen in the anterior midgut in vivo is the result of inputs from the CNS. However, the long timescale of responses to both SNP and serotonin suggests that these inputs serve a modulatory rather than an effective role.

REFERENCES


