EFFECT OF SUBSTANCE P ON THE LOWER ESOPHAGEAL SPHINCTER OF THE OPOSSUM

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Since the original observations by Von Euler and Gaddum, considerable interest has developed regarding the role of substance P in smooth muscle function. The purpose of the present investigation was to evaluate the effect of intravenously administered substance P on the in vivo motor function of the lower esophageal sphincter (LES). Intraesophageal pressures were monitored by an assembly of polyvinyl catheters attached to pressure transducers and a recorder. The catheters were continuously perfused with bubble-free water. Administration of 5, 10, 25, 50, and 100 ng per kg of substance P stimulated the LES, respectively, 16, 32, 57, 147, and 169% above control values. Tetrodotoxin, phentolamine, hexamethonium, methysergide, and bilateral cervical vagotomy did not alter the response of the LES to substance P. Atropine in 40-, 250-, and 500-μg per kg doses significantly but partially inhibited the response of the LES to substance P. It is concluded that substance P is a potent stimulant of the LES. The stimulatory effect of substance P may involve both cholinergic muscarinic and noncholinergic mechanisms. It is conceivable that substance P may be a modulator of LES pressure, although the exact physiological significance is not clear at the present time.

Forty-five years ago, Von Euler and Gaddum discovered the presence of a substance in the extracts from equine intestine and brain. This was named substance P. During the last four decades considerable information has been obtained regarding the chemical nature of substance P and its biological effects. Substance P has been shown to be present in high concentrations in the central nervous system. In view of the fact that substance P is present in higher concentration in the dorsal rather than the ventral roots of the spinal cord, it has been suggested that substance P might act as a neurotransmitter in primary sensory neurons. Substance P has been implicated in neuromuscular transmission in guinea pig ileum. An interesting finding reported by Ehrenpreis and Pernow is that substance P was lacking in the aganglionic segment of colon in Hirschsprung’s disease, indicated the possible clinical relevance of substance P. Although studies dealing with gastrointestinal and other smooth muscle preparations have been published, the effects of substance P on the esophagus have not been reported. The purpose of the present study was to evaluate the possible stimulatory effect of substance P on the lower esophageal sphincter (LES) pressure, and to study the mechanism of its stimulatory response.

Methods

Studies were performed on 35 adult opossums of either sex. The animals weighed from 1.7 to 3.3 kg and were anesthetized with barbital sodium, 200 mg per kg of body weight, injected intraperitoneally. Each experiment lasted for 5 to 8 hr during which time the animals did not usually need additional anesthesia. When necessary, small doses of barbital were injected intravenously to maintain anesthesia at a constant level. Intraluminal pressures in the LES and the body of the esophagus were monitored with a water-filled continuously perfused assembly of intraluminal catheters having three recording sites, at a distance of 2 cm. Each catheter (inner diameter, 0.86 mm; outer diameter, 1.17 mm) had a side opening and all were continuously perfused with bubble-free water with a constant infusion pump (Harvard Apparatus Co., Millis, Mass.). An infusion rate of 0.12 ml per min was utilized for all experiments. The catheter assembly was passed through the mouth of the animal until all openings were in the stomach. Subsequently, the catheter assembly was withdrawn slowly, 0.25 cm at a time. The LES was localized and the point at which the sphincter pressure was highest was noted. Throughout the experiment this position was strictly maintained. On occasions when the catheter assembly either moved proximally or distally, it was replaced in the previously noted position. The external jugular vein was exposed and catheterized for intravenous injections. A constant infusion of saline (0.15 ml per min) was utilized to keep the intravenous catheter patent. All drugs were injected intravenously except tetrodotoxin. Bilateral cervical vagotomy was carried out as described earlier. Tetrodotoxin was injected after laparotomy in the wall of the esophagus at the gastroesophageal junction. In some experiments the catheter assembly was fixed at the...
Imm. per kg of substance Hg and geal sphincter (LIB) to an intravenous bolus administration of substance P. hexamethonium on the response of the LES substance P, 50 ng per kg. adrenergic Q receptors and ganglionic transmission, we evaluated the effect of adrenergic cy receptor blocking agent phentolamine and ganglionic blocking agent hexamethonium on the response of the LES to 50 ng per kg of substance P (table 1). The per cent increases in LES pressure before and after atropine (40 µg per kg) injection of 50 ng per kg of substance P were 167.6 ± 15.9 (SE) and 161.8 ± 12.1 (SE) (P > 0.5). Corresponding values with 100 ng per kg of substance P were 163.8 ± 14.6 (SE) and 157.0 ± 13.6 (SE) (P > 0.5). Similarly, the per cent increases in LES pressure before and after hexamethonium (10 mg per kg) administration with 50 ng per kg of substance P were 167.6 ± 15.9 (SE) and 161.8 ± 12.1 (SE) (P > 0.5). Corresponding values with 100 ng per kg of substance P were 163.5 ± 7.2 (SE) and 179.1 ± 9.0 (SE) (P > 0.5).

Effect of atropine on the response of the LES to intravenous administration of substance P. To evaluate the possible participation of cholinergic muscarinic receptors in the response of the LES to substance P, we tested the effect of atropine. While the catheter position in the esophagus was controlled but not pinned in the esophagus, atropine (40 µg per kg) significantly antagonized the response of the LES to 25, 50, and 100 ng per kg of substance P (fig. 3). The per cent increases in LES pressure with 25, 50, and 100 ng per kg of substance P before and after atropine (40 µg per kg) were 57.4 ± 8.4 (SE) and 32.7 ± 5.1 (SE) (P < 0.02), 147.5 ± 9.1 (SE) and 78.2 ± 4.7 (SE) (P < 0.001), and 169.0 ± 13.3 (SE) and 82.5 ± 7.5 (SE) (P < 0.001), respectively. In order to avoid any influence of longitudinal movement of the catheter along the long axis of the esophagus, we repeated the study with the catheter assembly pinned in the wall of the esophagus in the gastroesophageal junction. The effects of 25 and 500 µg per kg of atropine on the response of the LES to intravenous administration of 50 and 100 ng per kg of substance P are shown in table 2. The effect of substance P was significantly antagonized by all doses of atropine even after the catheter assembly was pinned in the esophagus.

Effect of methysergide on the response of the LES to intravenous injection of 50 ng per kg of substance P were 155.5 ± 13.7 (SE) and 149.0 ± 14.1 (SE) (P > 0.5). Corresponding values with 100 ng per kg of substance P were 163.8 ± 14.6 (SE) and 157.0 ± 13.6 (SE) (P > 0.5). Similarly, the per cent increases in LES pressure before and after injection of 10 mg per kg of substance P were 165.0 ± 15.9 (SE) and 161.8 ± 12.1 (SE) (P > 0.5). Corresponding values with 100 ng per kg of substance P were 163.5 ± 7.2 (SE) and 179.1 ± 9.0 (SE) (P > 0.5).

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of potentiation of the response of the LES to substance P. The per cent increases in LES pressure before and after methysergide administration are shown in table 1. The per cent increases in LES pressure before and after methysergide (200 μg per kg) with intravenous bolus injection of substance P were 164.8 ± 6.0 (SE) and 164.1 ± 14.4 (SE). Corresponding values after 100 ng per kg of substance P were 187.9 ± 9.0 (SE) and 191.3 ± 11.0 (SE).

Effect of extrinsic and intrinsic denervation on the response of the LES to substance P. To evaluate the role of the vagus nerves in this regard, we tested the effect of bilateral cervical vagotomy on the response of the LES to substance P in 3 animals. Before vagotomy, the per cent increases in LES pressure in response to intravenous administration of 50 and 100 ng per kg of substance P were, respectively, 173.3 ± 18.0 (SE) n = 9, and 187.5 ± 11.0 (SE) n = 8. After bilateral cervical vagotomy, corresponding values with 50 and 100 ng per kg of substance P were 172.6 ± 16.2 (SE) n = 10 (P > 0.5) and 188.1 ± 10.6 (SE) n = 7 (P > 0.5), respectively. The influence of tetrodotoxin on the response of the LES to substance P was evaluated in 4 animals. Tetrodotoxin was injected locally in the gastroesophageal junction. The catheter assembly was pinned in the esophagus to avoid movement. Tetrodotoxin was administered in gradually increasing doses until LES relaxation in response to balloon distention was abolished. The dose of tetrodotoxin varied between 15 to 45 μg per kg. The per cent increases in LES pressure after intravenous administration of 100 ng per kg of substance P was 186.2 ± 12.0 (SE) before (n = 7) and 189.1 ± 13.0 (SE) after (n = 7) the local injection of tetrodotoxin. The difference was not statistically significant (P > 0.5).

Discussion

The present study demonstrated the very high order of potency of substance P with regard to its stimulatory effect on the LES. The stimulatory effect of substance P has several interesting aspects. First, atropine inhibited the stimulatory effect, although increasing the doses of atropine from 40 μg per kg to 250 and 500 μg per kg did not substantially increase the inhibitory effect. Because atropine inhibition of the effect of substance P was observed even after the catheter assembly was pinned in the esophagogastric junction, it is unlikely that any longitudinal movement of the catheter along the long axis of the esophagus was responsible for the decrease in response. Cholinergic muscarinic receptors are stimulatory to the LES. One way to explain the effect of substance P is that part of the response is mediated via muscarinic cholinergic receptors. Muscarinic cholinergic receptors in the gastrointestinal tract are located in the autonomic ganglia, the postganglionic parasympathetic neuron, as well as on the smooth muscle itself.  

In view of the fact that tetrodotoxin did not alter the response of the LES to substance P, it is unlikely that the effect is mediated neurally. However, a nonspecific release of acetylcholine similar to tyramine-induced release of norepinephrine cannot be excluded. In addition, the effect of atropine may also be explained as nonspecific depression of LES smooth muscle. It is likely that, if muscarinic receptors are involved in the response of the LES to substance P, the receptors are located on the smooth muscle of the LES. Although previous publications indicated that the effect of substance P is independent of cholinergic muscarinic transmission, some recent work indicates to the contrary. Hedqvist and Von Euler, dealing with longitudinal muscle of distal ileum, observed that transmural stimulation of the muscle (2 Hz, 1 to 2 pulses, 1 msec, supramaximal voltage) caused a twitch-like contraction blocked by atropine and regularly increased by \( 10^{-10} \) M concentration of substance P. Although atropine inhibited the response of the LES to substance P, it by no means abolished the response altogether. In fact, despite 250 and 500 μg per kg of atropine administration, substance P stimulated the LES between 80 to 90% above the control values. This indicates that a major part of the effect of substance P is mediated via a

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**Table 1. Per cent increases in lower esophageal sphincter pressure after administration of substance P. Effect of pharmacological antagonists and vagotomy.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>After blockade</th>
<th>Control</th>
<th>After blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phentolamine (2 mg/kg)</td>
<td>155.5 ± 13.7</td>
<td>149.9 ± 14.1</td>
<td>163.8 ± 14.6</td>
<td>157.0 ± 13.4</td>
</tr>
<tr>
<td>Hexamethonium (10 mg/kg)</td>
<td>167.6 ± 15.9</td>
<td>161.8 ± 12.1</td>
<td>183.5 ± 7.2</td>
<td>179.1 ± 9.0</td>
</tr>
<tr>
<td>Methysergide (200 μg/kg)</td>
<td>164.8 ± 6.0</td>
<td>164.1 ± 14.4</td>
<td>187.9 ± 9.0</td>
<td>191.3 ± 11.0</td>
</tr>
<tr>
<td>Bilateral cervical vagotomy</td>
<td>163.3 ± 18.0</td>
<td>172.6 ± 16.2</td>
<td>187.5 ± 11.0</td>
<td>188.1 ± 10.6</td>
</tr>
</tbody>
</table>

* Each number represents mean ± SE of 6 to 10 observations in 4 animals.
noncholinergic mechanism. Furthermore, hexamethonium, a compound that blocks transmission in autonomic ganglia without producing any preceding or concomitant change in the membrane potentials of ganglion cells, did not modify the response of the LES to substance P, indicating that the mechanism of stimulation is unlikely to involve postsynaptic cholinergic nicotinic receptors at the ganglia. Stimulation of the sensory end of the cut end of the vagus nerve stimulated the LES in one study. However, because bilateral cervical vagotomy did not alter the response of the LES to substance P, it seems unlikely that the response could be mediated via the vagal centers in the brain. It has been suggested that the substance P-like immunoreactive endocrine-like cells are identical with enterochromaffin cells, which are the major source of 5-hydroxytryptamine in the digestive system. Thus, an interaction between substance P and 5-hydroxytryptamine is conceivable. In the LES 5-hydroxytryptamine produces several different effects. It causes contraction of the LES by a direct action on the muscle and also by stimulation of cholinergic excitatory neurons. Because methysergide did not alter the response of the LES to substance P, it is unlikely that the stimulatory effect of substance P is mediated via 5-hydroxytryptamine receptors. Our observation in this regard is similar to those in previous publications.

The concentration of substance P-like immunoreactivity has been extensively studied by Nilsson and Brodin. Highest concentration of substance P was found in the duodenum of dog and cat. Substance P immunoreactivity has been localized to nerve fibers forming a dense network around ganglionic cell bodies of the myenteric plexus or running singly in between smooth muscle cell layers. Relatively low concentrations were observed in extracts from the tongue, esophagus, and stomach. There was considerable species variability in the tissue concentrations. Although the dog distal esophagus had only 0.4 ng per g of tissue of substance P, the distal mouse esophagus had 5.7 ng per g of tissue of substance P. No reports are available regarding substance P content of the opossum esophagus.

Table 2. Effect of atropine on the response of the lower esophageal sphincter (LES) to substance P while the catheter assembly was pinned in the esophagus in 5 animals

<table>
<thead>
<tr>
<th>Doses of substance P</th>
<th>Before atropine</th>
<th>After 40 μg/kg atropine</th>
<th>After 250 μg/kg atropine</th>
<th>After 500 μg/kg atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ng/kg</td>
<td>166.0 ± 10.1</td>
<td>90.7 ± 19.1</td>
<td>59.0 ± 10.1</td>
<td>60.0 ± 13.5</td>
</tr>
<tr>
<td>n = 9</td>
<td></td>
<td>n = 8 (P &lt; 0.005)</td>
<td>n = 11 (P &lt; 0.001)</td>
<td>n = 11 (P &lt; 0.001)</td>
</tr>
<tr>
<td>100 ng/kg</td>
<td>175.4 ± 11.9</td>
<td>94.1 ± 15.2</td>
<td>65.5 ± 9.1</td>
<td>56.8 ± 14.0</td>
</tr>
<tr>
<td>n = 9</td>
<td></td>
<td>n = 7 (P &lt; 0.001)</td>
<td>n = 11 (P &lt; 0.001)</td>
<td>n = 10 (P &lt; 0.001)</td>
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</table>

* Values are given as means ± se.

stimulates both the synthesis and utilization of dopamine, norepinephrine, and 5-hydroxytryptamine. There is evidence that the dopaminergic cell bodies or dendrites in the substantia nigra receive a rich supply of substance P-carrying nerve terminals. In the esophagus, a dopaminergic inhibitory mechanism has been demonstrated both in vivo as well as in vitro. Whether the stimulatory effect of substance P and the inhibitory effect of dopamine are related in some way is not known. Further studies are needed to clarify the exact role of substance P in relation to esophageal motor function.

REFERENCES

14. Daniel EE: Pharmacology of the gastrointestinal tract. In Hand-


