Neuronal release of endogenous dopamine from corpus of guinea pig stomach

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Shicijo, Kazuko, Yusaku Sakurai-Yamashita, Ichiro Sekine, and Kohtarou Taniyama. Neuronal release of endogenous dopamine from corpus of guinea pig stomach. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1044–G1050, 1997.—Neuronal release of endogenous dopamine was identified in mucosa-free preparations (muscle layer including intramural plexus) from guinea pig stomach corpus by measuring tissue dopamine content and dopamine release and by immunohistochemical methods using a dopamine antiserum. Dopamine content in mucosa-free preparations of guinea pig gastric corpus was one-tenth of norepinephrine content. Electrical transmural stimulation of mucosa-free preparations of gastric corpus increased the release of endogenous dopamine in a frequency-dependent (3–20 Hz) manner. The stimulated release of dopamine was prevented by either removal of external Ca2+ or treatment with tetrodotoxin.

Dopamine-immunopositive nerve fibers surrounding choline acetyltransferase-immunopositive ganglion cells were seen in the myenteric plexus of whole mount preparations of gastric corpus even after bilateral transection of the splanchic nerve proximal to the junction with the vagal nerve (section of nerves between the celiac ganglion and stomach). Damphetamine and sulpiride potentiated the stimulated release of acetylcholine release from mucosa-free preparations. These results indicate that dopamine is physiologically released from neurons and from possible dopaminergic nerve terminals and regulates cholinergic neuronal activity in the corpus of guinea pig stomach.

1 Neuronal release of endogenous dopamine from corpus of guinea pig stomach (10, 12), which contain considerable amounts of norepinephrine (NE) and smaller amounts of dopamine (7, 8). It is not clear whether dopamine exists solely as a precursor of NE or as a bioactive substance in its own right in gastric tissues. In this study, we report data to support the idea that dopamine is released from neurons, possibly dopaminergic neurons, in guinea pig stomach corpus and regulates activities of cholinergic neurons through dopamine receptors.

MATERIALS AND METHODS

Measurements of dopamine and NE in the tissues. Adult guinea-pigs of either sex, weighing 300–500 g, were separated into two groups. The first group was used as a control group, and the second group was treated with a bilateral transection of the splanchic nerve proximal to the junction with the vagal nerve (section of nerves between the celiac ganglion and stomach) (20). 7 days before experiments were performed. The guinea pigs were killed by cervical dislocation and the stomach was immediately excised. Segments of tissue were obtained from the stomach corpus in two groups of guinea pigs. The gastric corpus was scraped with a glass slide and separated into the mucosal layer and the muscle layer including the intramural plexus (mucosa-free preparation). Each preparation was homogenized with an ultrasonic probe homogenizer in 500 µl of solution containing 0.4 N HClO4, 5.3 mM Na2S2O5, 1.4 mM EDTA, and 3,4-dihydroxybenzylamine (50 ng/ml) as the internal standard. Homogenates were centrifuged at 15,000 revolutions per minute (4°C, 15 min), and isolation of dopamine and NE from the supernatant was accomplished by absorption with alumina (50 mg) in 2 ml of 0.5 M triis(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 8.6). Dopamine and NE were then eluted by 150 µl 0.1 N HClO4. A 25-µl aliquot of the HClO4 eluent was injected into a high-performance liquid chromatograph with an electrochemical detector (HPLC-ECD).

Measurements of dopamine release. The stomach corpus was cut into strips ~5 × 15 mm, in a circular fashion. The strips were immediately separated into a mucosal layer and a muscle layer including the intramural plexus of the gastric corpus. The preparation of muscle layer including the intramural plexus was mounted in an apparatus and superfused at 37°C at a flow rate of 1 ml/min with Krebs solution of the following composition (in mM): 118 NaCl, 4.8 KCl, 2.5 CaCl2, 1.19 MgSO4, 25.0 NaHCO3, 1.18 KH2PO4, and 11 glucose, containing 0.1 mM pargyline and 0.01 mM ascorbic acid and gassed with 95% O2-5% CO2. Another preparation obtained by the same procedure was frozen, cut into sections 20 µm thick on a cryostat, and stained with hematoxylin and eosin for microscopic examination. Experiments were started 60 min after the superfusion. When the spontaneous release of dopamine had approached a plateau. The preparations were stimulated by two parallel platinum electrodes at parameters of 1-ms duration, 15-V intensity at various frequencies for 3 min at 70, 95, and 120 min after the superfusion. The superfusate was continuously collected into a test tube containing 0.6 ml 1 N HClO4, 0.5 ng 3,4-dihydroxybenzy-
Dopamine release from stomach

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Dopamine was released from the muscle layer including the intramural plexus of the gastric corpus. NE content was 195 ± 22 ng/g wet wt (n = 5) in the muscle layer including the intramural plexus of the gastric corpus. NE content was 195 ± 22 ng/g wet wt (n = 5) in the muscle layer including the intramural plexus of the gastric corpus. The ratio of dopamine to NE was 0.17 in the mucosal layer and 0.11 in the muscle layer including intramural plexus.

Release of endogenous dopamine from the muscle layer including the intramural plexus of the gastric corpus. The spontaneous release of endogenous dopamine from the muscle layer including the intramural plexus of the gastric corpus remained steady during the course of each 140-min experiment. The amount of spontaneously released dopamine was 0.122 ± 0.011 ng·min⁻¹·g wet wt⁻¹, and the fractional rate (amount in superfusate/amount in tissue) of spontaneously released dopamine was 0.00127 ± 0.00007/min (mean ± SE for 38 determinations).

Electrical transmural stimulation (15-V intensity, 1-ms pulse duration) for 3 min increased the release of dopamine and NE contents in gastric corpus. Dopamine content was 32.9 ± 1.9 ng/g wet wt (n = 5) in the mucosal layer and 95.8 ± 23.2 ng/g wet wt (n = 5) in the muscle layer including the intramural plexus of the gastric corpus. NE content was 195 ± 22 ng/g wet wt (n = 5) in the muscle layer and 879 ± 77 ng/g wet wt (n = 5) in the muscle layer including the intramural plexus.
endogenous dopamine above the spontaneous release noted just before the stimulation, in a frequency (3–20 Hz)-dependent manner (Table 1), although electrical stimulation at a frequency of 1 Hz did not significantly increase the release of dopamine. The electrically (15-V intensity, 1-ms pulse duration, at a frequency of 10 Hz) induced increase in dopamine release was markedly reduced by either treatment with tetrodotoxin (TTX) (Fig. 1A) or removal of Ca\(^{2+}\) from the superfusion medium (Fig. 1B).

Immunohistochemistry of dopamine in whole mount preparations from the gastric corpus. Immunostaining with specific antiserum against dopamine was studied in whole mount preparations from guinea pig stomach corpus. In all 18 preparations studied, a network of dopamine-immunopositive fine varicose fibers was found to surround the nonimmunoreactive ganglion cells within the myenteric plexus of the gastric corpus (Fig. 2A). A fairly dense immunostaining of nerve fibers was prominent in the primary plexus running from ganglion to ganglion, without leaving the plane of the plexus. Dopamine-immunopositive varicose nerve fibers were also observed around the blood vessels (data not shown). The same pattern of dopamine immunostaining was seen in parallel experiments using the antidopamine serum adsorbed with NE, whereas no immunostaining was seen when antidopamine serum adsorbed with dopamine or nonimmune serum was used. The immunopositive nerve cell bodies (~1 cell per 5 ganglia) were seen in the periphery of the myenteric ganglia (Fig. 2B). They were generally spheroidal in shape and gave rise to two thick processes within the myenteric plexus. The population of cell bodies immunoreactive to dopamine was 0.19 ± 0.07 cells/ganglion (mean ± SE, 53 ganglia/6 preparations).

Tissue contents and Immunohistochemistry of dopamine after bilateral transection of the splanchic nerve proximal to the junction with the vagal nerve. Seven days after bilateral transection of the splanchic nerve proximal to the junction with the vagal nerve (section of nerves between the celiac ganglion and stomach), dopamine content was 33.3 ± 6.8 ng/g wet wt (n = 5) in the mucosal layer and 81.9 ± 8.2 ng/g wet wt (n = 5) in the muscle layer including the intramural plexus, indicating that dopamine content in these layers was not altered by section of nerves between the celiac ganglion and stomach.

In the nontreated animals, the major patterns of catecholaminergic innervation in the stomach detected by fluorescence histochemistry were as described by Furness and Costa (10). A network of fine varicose fibers was found to surround the nonfluorescent ganglion cells of the myenteric plexus (Fig. 3A). After section of nerves between the celiac ganglion and stomach, catecholamine fluorescence was virtually abolished from the tertiary plexus and the muscle layers, and fewer fluorescent fibers were observed in the myenteric plexus (Fig. 3B), whereas no conspicuous changes in dopamine-immunopositive nerve fibers were seen in the myenteric plexus of the gastric corpus (Fig. 3C). Double staining for dopamine and CAT demonstrated that a network of dopamine-immunopositive fine varicose fibers closely encircled the CAT-positive ganglion cell bodies within the myenteric plexus, even after section of nerves between the celiac ganglion and stomach (Fig. 3C).

Effects of dopamine receptor antagonists and dopamine on the electrically stimulated outflow of ACh. Electrical transmural stimulation (15 V, 1 ms) at 10 Hz evoked release of endogenous dopamine from preparations consisting of the outer muscle layers and myenteric plexus. The possible effects of this endogenous

<table>
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<tr>
<th>Frequency, Hz</th>
<th>Amount of Stimulated Release, ng/5 min/g wet wt</th>
<th>Fractional Rate, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.034 ± 0.038</td>
<td>0.0013 ± 0.00014</td>
</tr>
<tr>
<td>3</td>
<td>0.351 ± 0.122</td>
<td>0.0020 ± 0.00019</td>
</tr>
<tr>
<td>5</td>
<td>0.595 ± 0.131</td>
<td>0.0025 ± 0.00026</td>
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<tr>
<td>10</td>
<td>1.056 ± 0.166</td>
<td>0.0038 ± 0.00031</td>
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<tr>
<td>20</td>
<td>1.227 ± 0.234</td>
<td>0.0038 ± 0.00039</td>
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Values are means ± SE for 5 guinea pigs. Amount of stimulated release of dopamine represents value obtained by subtraction of spontaneous release immediately before electrical stimulation from release during stimulation. Fractional rate was obtained by dividing the amount of dopamine in the superfusate by the respective amount of dopamine in the tissue. *Significantly different from spontaneous release value (P < 0.05 by Dunnett’s t-test).
dopamine on the release of $[^3H]ACh$ were examined using specific dopamine receptor antagonists. Electrical transmural stimulation (15 V, 1 ms) at 10 Hz for 1 min increased the outflow of $[^3H]$ from the muscle layer including intramural plexus preloaded with $[^3H]$choline. Applications of $D_2$ dopamine receptor antagonists (sulpiride and domperidone) at concentrations of 100 nM to 1 µM and $\alpha_2$-adrenoceptor antagonist (yohimbine) at concentrations of 10 nM to 1 µM significantly potentiated the electrically stimulated outflow of $[^3H]$ (Fig. 4A), with no effect on spontaneous outflow. Even in the presence of 0.1 µM yohimbine, sulpiride and domperidone also significantly potentiated the electrically stimulated outflow of $[^3H]$ (Fig. 4B). Sulpiride and domperidone reversed the inhibitory action of dopamine (1 µM) on the stimulated outflow of $[^3H]$ (Fig. 4C).

**DISCUSSION**

The guinea pig gastric corpus contains measurable concentrations of dopamine: the concentration in the outer muscle layers (which include the intramural plexus) was higher than that in the mucosal layer. The ratio of dopamine to NE in the muscle layer including myenteric plexus was 0.11, similar to that in the smooth muscle/myenteric plexus preparation of mouse stomach fundus (7). In the generally noradrenergically innervated tissues, dopamine and NE are measurable in a relatively constant ratio of dopamine to NE within a species, and a high ratio of dopamine to NE in any tissue suggests that dopamine is not only the precursor of NE but is also a bioactive substance (4, 19). Thus dopamine contained in the muscle layer including the intramural plexus of the gastric corpus is thought to act as a bioactive substance, although in the stomach the abundance of catecholaminergic innervations has been demonstrated by fluorescent glyoxylic acid histochemistry (10, 12), and it is accepted that the neurons are NE-containing sympathetic adrenergic neurons.

The amount of dopamine released spontaneously from the muscle layer including intramural plexus of gastric corpus was approximately 0.13% per minute of the content in the tissue. This value is similar to that released from the central dopaminergic neurons (25, 34). Electrical transmural stimulation increased the release of endogenous dopamine above the spontaneous release from the muscle layer including intramural
plexus of gastric corpus. When the properties of the stimulated release of dopamine were determined, the stimulated release was found to be TTX sensitive and external Ca\(^{2+}\) dependent. TTX blocks neuronal conduction (24), and external Ca\(^{2+}\) is necessary for the exocytotic release of most neurotransmitters from nerve terminals (27). Thus these findings support the concept that dopamine is released in response to stimulation from a neuronal component of the corpus of the guinea pig stomach. The possibility that stimulation of adrenergic nerves results in the release of dopamine as well as NE cannot be completely excluded.

Immunohistochemical studies using dopamine antibody demonstrated the presence of dopamine-immunopositive varicose nerve fibers within the myenteric plexus of the guinea pig stomach corpus. Dopamine-immunopositive nerve fibers were prominent in the primary plexus running from ganglion to ganglion without leaving the plane of the plexus, particularly with no projection to the muscle layer, being different in density within the myenteric plexus and in distribution

**Fig. 3.** Catecholamine histochemistry (A and B) and double staining of dopamine and choline acetyltransferase (CAT) (C) within myenteric plexus in mucosa-free whole mount preparation of corpus of guinea pig stomach. A: normal animal. B and C: animal after bilateral transection of splanchnic nerve proximal to junction with vagal nerve (section of nerves between celiac ganglion and stomach). C: dopamine-immunopositive fine varicose fibers (brown) closely encircled CAT-immunopositive ganglion cells (blue, arrows) in myenteric plexus. Scale bar, 50 µm.

**Fig. 4.** Effects of dopamine receptor antagonists on electrically stimulated release of \(^{3}H\) acetylcholine (ACh) from mucosa-free preparation of corpus of guinea pig stomach. A: potentiation of electrically stimulated release of \(^{3}H\)ACh by yohimbine, sulpiride, and domperidone. B: potentiation of electrically stimulated release of \(^{3}H\)ACh in presence of yohimbine (0.1 µM) by sulpiride and domperidone. C: antagonism by sulpiride and domperidone on inhibitory effect of dopamine on electrically stimulated release of \(^{3}H\)ACh. Dopamine (1 µM), yohimbine, sulpiride, and domperidone were applied 2 min, 10 min, 10 min, and 10 min before and during electrical stimulation (1-ms duration, frequency 10 Hz), respectively. *Significantly different from value in absence of antagonists (control) in A and B and value of dopamine effect in absence of antagonists in C (P < 0.05 by Dunnett's t-test).
of nerve fibers from the findings demonstrated by catecholamine fluorescence histochemistry in the present study and previously (10, 12) and by immunohistochemistry using tyrosine hydroxylase antibody (3, 22, 31). The neurons containing both tyrosine hydroxylase and dopamine-β-hydroxylase reflect the majority of NE-containing neurons, whereas the neurons containing tyrosine hydroxylase, but not dopamine-β-hydroxylase, may be dopaminergic neurons. Dopaminergic nerve fibers have been suggested to be present in the rat stomach, based on the findings that the immunoreactive nerve fibers to tyrosine hydroxylase, but not to dopamine-β-hydroxylase, were detected in the myenteric plexus of the rat stomach by double staining for tyrosine hydroxylase and dopamine-β-hydroxylase (3). After section of nerves between the celiac ganglion and stomach, dopamine contents in the mucosal layer and the muscle layer including intramural plexus were not altered, and no conspicuous changes in dopamine-immunoreactive nerve fibers in the plexus were seen. However, catecholamine fluorescence was virtually abolished from muscle layers and fewer fluorescent fibers were observed in the plexus. Gastric NE is contained mainly in sympathetic fibers derived from the celiac and superior mesenteric ganglia (8, 20). If dopamine is a precursor of NE in the sympathetic neurons, section of nerves between the celiac ganglion and stomach may reduce gastric dopamine content and dopamine-immunoreactive nerve fibers. Double staining for dopamine and CAT demonstrated that a network of dopamine-immunoreactive fine varicose fibers closely encircled the CAT-positive ganglion cell bodies within the myenteric plexus, even after section of nerves between the celiac ganglion and stomach. Thus dopaminergic nerve fibers innervate the myenteric plexus of the guinea pig stomach, and the dopamine released from these nerve terminals by nerve stimulation may act on the enteric cholinergic neurons.

Dopamine-immunoreactive nerve cell bodies with two long thick projections were seen in the periphery of myenteric ganglia. Although it was not possible to observe the full extent of the emergent processes and therefore define their class exactly, the stained cells with two long processes appear to resemble the pseudouni-axonal multiaxonal type II neurons in the intestine, according to the morphological features classified into eight types of enteric neurons (33), whereas Dogiel type II-AH/type II neurons have been shown to be absent in the stomach (32). It is difficult to interpret characteristics of the dopamine-immunoreactive nerve cell bodies, because no tyrosine hydroxylase-immunoreactive neurons have been detected in the myenteric plexus of the guinea pig stomach (22, 29, 31), and in fact the nerve cell bodies immunonegative to tyrosine hydroxylase and immunoreactive to dopamine-β-hydroxylase have been demonstrated (29, 31). Thus the present findings suggest the possible presence of dopaminergic intrinsic neurons in the enteric nervous system of the guinea pig stomach as interneurons or nonmotor sensory neurons (30), although it cannot be excluded that the neurons may be capable of taking up dopamine but may not synthesize it. In the guinea pig small intestine, intrinsic amine-handling neurons have been shown, although these cells did not contain tyrosine hydroxylase and dopamine-β-hydroxylase (11).

The source of dopamine-immunoreactive nerve fibers within the myenteric plexus is thus possibly 1) the dopaminergic intrinsic neurons detected in the present study; 2) the vagal efferent, parasympathetic fibers originating from the dorsal nucleus of vagus nerve, which contains neurons immunoreactive to tyrosine hydroxylase but not to dopamine-β-hydroxylase (2); 3) the vagal afferent fibers of the nodose ganglion projecting to the stomach, which exhibit tyrosine hydroxylase immunoreactivity and aromatic l-amino acid decarboxylase immunoreactivity but not dopamine-β-hydroxylase immunoreactivity (16); and 4) a subset of celiac ganglion sympathetic neurons, which contain neurons immunoreactive to tyrosine hydroxylase but not to dopamine-β-hydroxylase.

Activation of D₂ dopamine receptor has been reported to inhibit the release of ACh from cholinergic neurons in the stomach (17). Sulpiride and domperidone are D₂ dopamine receptor antagonists, although domperidone exerts an inhibitory effect on cholinesterase, and both antagonists were found to potentiate the release of ACh stimulated by electrical stimulation under conditions that stimulated the release of endogenous dopamine. Thus the blockade of the D₂ dopamine receptor with sulpiride or domperidone prevented the inhibitory effect of endogenous dopamine released by electrical stimulation on the cholinergic neurons, resulting in potentiation of the release of ACh. The dopamine-induced inhibition of stimulated release of ACh was reversed by sulpiride and domperidone, which is similar to the finding noted previously (17). As well, activation of α₂-adrenoceptor has been shown to inhibit the release of ACh from the cholinergic neurons of the stomach (1). The potentiating effects of D₂ dopamine receptor antagonists were obtained even when the α₂-adrenoceptor was blocked by yohimbine. Thus the target of dopamine released from neurons may be the cholinergic neurons, and dopamine inhibits cholinergic neuronal activity.

Dopamine may be present in the corpus of the guinea pig stomach not merely as a precursor of NE, but may act through specific dopamine receptors as well. The present study using biochemical, physiological, and immunohistochemical methods showed neuronal release of dopamine from the gastric corpus and suggests that dopamine may play a physiological role in the control of gastric motility through modulation of cholinergic neuronal activity.

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REFERENCES

1. Alberts, P. Mechanisms of facilitation and muscarinic or α-adrenergic inhibition of acetylcholine and noradrenaline secre-
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