cles (*2*, *13*, *14*). However, the CNS, including the cerebrum, cerebellum, medulla oblongata, and spinal cord, is spared this K^+ decrease, presumably as a result of the maintenance of normal K^+ levels in the cerebrospinal fluid by the choroid plexus (*15*). Also, heart and liver can maintain their normal $[Na]_i$ and $[K]_i$ values even though these organs are equally exposed to plasma hypokalemia (*16*). Perhaps the CNS can influence the distribution of Na^+ and K^+ in the body by selectively inhibiting the pump in certain skeletal and smooth muscles during hypokalemia. At least in the initial stages of hypokalemia, such peripheral organs may serve as a reservoir for K^+ without seriously compromising their function. Consequently, a slow net release of K^+ from these organs may serve as a buffer between the animal's intake of K^+ and its plasma K^+ concentration, thus maintaining homeostasis.

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5. The values for $[Na]_i$ and $[K]_i$ in 30 fresh muscles in normal rats are (mean ± 1 standard deviation) 24.2 ± 3.2 and 134.1 ± 7.4 mmole per liter of fiber water, respectively [see Akaike (*2*)].
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Release of Immunoreactive Serotonin into the Lumen of the Feline Gut in Response to Vagal Nerve Stimulation

Abstract. *Immunoreactive serotonin was detected in the lumen of the proximal jejunum of food-deprived cats. During perfusion of this intestinal segment in vivo, there was a constant basal rate of intraluminal secretion of this amine. The rate of secretion was significantly increased during efferent electrical stimulation of the cut cervical vagal nerves. This stimulatory effect was not altered after bilateral adrenalectomy was performed in the same animals. A synchronous release of substance P into the gut lumen was also demonstrated during vagal stimulation. During the period of increased intraluminal secretion of immunoreactive serotonin, there was no demonstrable change in the portal or systemic blood levels of this amine.*

In the mammalian gastrointestinal tract, serotonin is largely stored within the enterochromaffin cells (EC) of the intestinal mucosa (*1*). Efferent electrical stimulation of the feline cervical vagal nerves in vivo decreases the amount of serotonin in individual EC cells prepared for fluorescence microscopy by the Hillarp-Falck technique for visualizing intracellular monoamines (*2*). This observation supports the concept of a vagal release of the amine from these cells (*2*). Furthermore, a direct innervation of EC cells has been shown at the ultrastructural level (*3*). Hypothetically, the released amine may be extruded through the basolateral membrane of these cells and diffuse into subepithelial capillaries or may be secreted into the gut lumen through the apical cytoplasmic processes, or perhaps both. Ultrastructural studies of vagally stimulated gut mucosa obtained from biopsies demonstrated signs of active exocytosis of the basolateral membrane but also indicated accumulation of electron-dense material in the most apical portions of the cells (*4*). Combined electron microscopy and autoradiography after injection of 5-[^{3}H]hydroxytryptophan (the serotonin precursor) demonstrated release of the radioactive tracer into the gut lumen in response to electrical vagal nerve stimulation (*5*). We have now established the presence of intraluminal serotonin in the feline proximal jejunum and determined the effect of nerve stimulation in vivo on the levels of this amine in the lumen and in the portal and femoral veins.

Six cats weighing 2.6 kg were deprived of food for 18 hours. The cats were anesthetized with chloralose (100 mg/kg, intravenously), and a 15-cm segment of the proximal jejunum was isolated by dividing the bowel but not the mesentery; the lumen was constantly perfused with saline (37°C) at a rate of 1.0 ml/min. Venous blood samples were drawn from heparinized Teflon catheters in the portal and left femoral veins, and the perfusates were collected every 5 minutes. The concentrations of serotonin were measured by a sensitive radioimmunoassay technique developed in our laboratory (*6*); extractions of serotonin from the blood samples and perfusates were carried out with the same technique (*6*). Recovery of both ^{3}H-labeled and unlabeled serotonin added to portions of the blood samples averaged 64.5 ± 1.9 percent and to portions of the perfusates averaged 91.2 ± 2.4 percent. High intraluminal levels of immunoreactive serotonin were detected (80 to 720 ng per 5 minutes) during a 15-minute washout period. After the washout, the basal rate of intraluminal serotonin secretion averaged 64 ± 12 ng per 5 minutes ($N = 6$). The presence of serotonin in the intestinal lumen was confirmed by high-performance liquid chromatography (*7*), which yielded comparable data.

After two such basal periods, bilateral efferent electrical stimulation (10 V, 5 msec, 8 to 10 Hz) of the cut cervical vagal nerves inserted into silver ring electrodes caused a rapid increase in the rate of secretion of the amine in all six cats (Table 1). The *t*-test for paired data was used to confirm that the 1.93 ± 0.35-fold increased rate of luminal secretion of serotonin during vagal stimulation was significantly ($P < .01$) higher than the basal rate. When stimulation was stopped, the rate of intraluminal secretion of immunoreactive serotonin abruptly returned to the basal value.

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During the period of stimulated luminal release, peripheral and portal venous concentrations of immunoreactive serotonin did not change significantly (Table 1). Failure to demonstrate increases in venous serotonin concentrations is neither artifactual nor related to platelet binding, since we have previously demonstrated that duodenal acidification (*8, 9*), a test meal (*6*), and serotonin infusion (*8*) all increase circulating concentrations of serotonin. The higher concentration of serotonin in the portal vein than in the peripheral vein is consistent with hepatic inactivation of this amine (*9*).

Since the stimulation procedure results in bradycardia and decreased systemic arterial blood pressure, three animals were subjected to adrenalectomy to exclude compensatory adrenal influence. Before adrenalectomy, stimulation resulted in an average luminal output of 138.3 ± 65.5 ng per 5 minutes. After adrenalectomy, stimulation resulted in an average release of 154.0 ± 62.7 ng per 5 minutes. This mean peak value was not significantly different from the values before adrenalectomy.

Since substance P and serotonin coexist within the same EC cells (*10*), and substance P is released into the feline antrum (*11*), concentrations of substance P in the perfusates from individual animals were determined with a radioimmunoassay that we have recently described (*12*). The recovery of substance P from portions of the perfusates averaged 80.0 ± 4.1 percent. Synchronous peaks of immunoreactive substance P and serotonin released during vagal stimulation were observed in one of these animals before and after adrenalectomy (Fig. 1). This release of substance P was confirmed in five additional animals; increases (above basal) at 5, 10, and 15 minutes averaged 29.8 ± 5.2, 24.4 ± 3.2, and 14.3 ± 4.7 pg per 5 minutes, respectively. Within 10 minutes after vagal stimulation, substance P secretion fell to the basal value, decreasing to 7.6 ± 1.5 pg per 5 minutes. The vagally stimulated luminal release of immunoreactive substance P was synchronous with that of serotonin. Thus, it seems likely that vagal nerve stimulation of the isolated gut segment activates similar mechanisms or a common release mechanism so that there is a simultaneous intraluminal secretion of the amine and the peptide into the jejunum.

Although immunoreactive serotonin is present in the gut lumen under fasting conditions, intraluminal serotonin release is substantially augmented by vagal nerve stimulation, without demonstrable changes occurring in venous blood levels. The use of the isolated jejunal segment excludes the effects of contractions and the influence of secretory products from the duodenum on the jejunal mucosa. The presence of a substance in the guinea pig ileum with serotonin-like biological activity has been demonstrated (*13*). However, our observation indicates that the luminal release mechanism is neurally mediated and may be of major importance.

Since blood samples were collected every 5 minutes, it is conceivable that a transient peak in serotonin concentrations in the portal circulation was missed. However, this is unlikely since vagal stimulation resulted in a plateau of luminal serotonin secretion. Furthermore, had there been a substantial increment in the concentration of endogenous serotonin in the portal circulation, we would have expected to recognize a parallel increase in systemic serotonin levels (*8*).

Our study indicates that a purely endocrine function of serotonin is doubtful; it probably has a neurocrine role, although the significance of intraluminal serotonin is not known. Bülbring and Lin (*13*) reported that serotonin added to the fluid passing through the lumen of the intestine stimulated peristalsis. Among other roles, the amine may exert trophic effects on the gastrointestinal mucosa, as has been demonstrated for intraluminal gastrin (*14*). A luminal serotonin receptor may be important in the mediation of potent actions of the amine on gastrointestinal motility and secretion in the postprandial response of the small intestine (*15*), especially since rapid degradation by intracellular monoamine oxidase may be avoided with luminal secretion. Our study challenges the classical endocrine role of the EC cell and also demonstrates a purely neurocrine mechanism for the simultaneous luminal secretion of an amine and a peptide hormone from the EC cell.

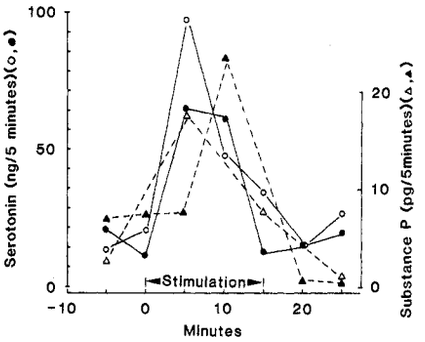

Fig. 1. Synchronous changes in immunoreactive serotonin (○ and ●) and substance P (△ and ▲) in intestinal perfusates in one cat during vagal nerve stimulation before (open symbols) and after (closed symbols) bilateral adrenalectomy.

Table 1. Serotonin levels in intestinal perfusates and in femoral and portal venous blood before, during, and after efferent stimulation of the cervical vagal nerves (10 V, 5 msec, 8 to 10 Hz) in the cat (N = 6). Values are means ± standard error.

Time (minutes)	Rate of intraluminal secretion (nanograms per 5 minutes)	Peripheral venous blood (ng/ml)	Portal venous blood* (ng/ml)
	Before stimulation		
−5	64 ± 12	229 ± 56	
0	66 ± 21	236 ± 49	377 ± 60
	During stimulation		
5	142 ± 37†	261 ± 45	430 ± 109
10	109 ± 30	354 ± 94	340 ± 70
15	124 ± 42†	200 ± 20	436 ± 73
	After stimulation		
20	72 ± 32	260 ± 60	423 ± 61
25	55 ± 16	208 ± 10	

*N = 4. †P < .01.

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