acid that forms readily from threonine and methionine. Homoserine, which can form either from cystathionine or more importantly by reductive deamination of canaline (*14*), is subject to a deamination that also forms 2-oxobutyric acid. While the above reactions have not been studied specifically in *C. brasiliensis*, their occurrence in insects is well documented and they represent reasonable pathways for the transfer of the ^{15}N isotope to the various amino acids of this seed-feeding insect. On the other hand, a glutamic acid-2-oxoglutarate aminotransfer is an integral aspect of leucine, isoleucine, lysine, and histidine biosynthesis. It is evident, therefore, that this nitrogen-transferring reaction does not support all the amino acid biosynthetic pathways operative in this seed predator.

Many, perhaps even all, of the metabolic capacities reflected in the catabolism and detoxification of canavanine and canaline by *C. brasiliensis* larvae (*14*) may emanate from the enzymes synthesized by the prokaryotic flora of the insect rather than the insect itself.

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5. [1,3-$^{15}N_2$]Urea (99 percent ^{15}N) was purchased from Stohler Isotope Chemical Co., Azusa, Calif. Its purity and ^{15}N content were confirmed by mass spectrometry.
6. Stirring the resin overnight at 3°C with a large excess of 0.5*N* ammonia failed to yield additional amino acids.
7. Automated amino acid analysis of pooled fractions 1 to 35, 36 to 69, 111 to 125, and 126 to 140 failed to reveal the presence of appreciable ninhydrin-positive material.
8. Automated amino acid analysis was conducted with the lithium citrate physiological buffer system of Durram Chemical Co. (Dionex Corp.). The process involves the sequential application of five buffers and requires 5 hours to complete. The amino acid derivatives used for gas chromatography–mass spectrometry were prepared by the method of D. L. Stalling and C. W. Gehrke [*Biochem. Biophys. Res. Commun.* **22**, 329 (1966)]. Mass fragment identification was based on the work of E. Gelpi *et al.* [*J. Chromatogr. Sci.* **7**, 604 (1969)] and R. E. Summons *et al.* [*Anal. Chem.* **46**, 582 (1974)]. Gas chromatography–mass spectrometry analyses were conducted with a Hewlett-Packard model 5985-A mass spectrometer operated in the electron impact mode with an accelerating voltage of 70 eV and source temperature of 150°C.
9. The small relative abundance of mass spectral fragments containing nitrogen prevented the determination of the ^{15}N content of phenylalanine, tyrosine, and tryptophan. Ornithine and canavanine were not assayed since neither ^{15}N-labeled standards for relevant mass spectral literature exist for the compounds. ^{15}N-Labeled canaline was not assayed since either during derivatization or the actual gas chromatography–mass spectrometry process it was degraded.
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Cholinergic Agonists Induce Vectorial Release of Serotonin from Duodenal Enterochromaffin Cells

Abstract. *Serotonin-containing enterochromaffin cells in the rabbit duodenal mucosa span the tissue contacting both the luminal and serosal sides. When the serosal surface is stimulated with carbachol in vitro, serotonin is secreted on the serosal side but not the mucosal side. Carbachol added to the luminal side is ineffective. Atropine but not hexamethonium blocks the effect of carbachol. Acetylcholine on the serosal surface also stimulates serotonin release on the serosal side. These findings indicate that enterochromaffin cells possess on their serosal surfaces muscarinic receptors that mediate vectorial release of serotonin when activated by cholinergic agonists.*

The enterochromaffin cells in the gastrointestinal mucosa contain a large proportion of the body's store of serotonin (*1*). In spite of this, little is known about the regulation of these cells or the precise role of the serotonin they release. The enterochromaffin cells typically span the mucosa. In general, the apical pole of each cell borders the intestinal lumen and is striated with microvilli (*2*). The polymorphous secretory granules that contain the amine are most commonly found in the widened base of the cell but occasionally are found in the apical portion (*3*). Presumably, dietary stimuli induce responses in the apical portion (*4*) and endocrine or nerve-derived factors induce responses in the basal portion (*5*). In vivo studies indicate that the cells have the ability to secrete serotonin from their apical or basal sides, leading to increases in either luminal (*4*) or blood (*5*) concentrations of serotonin. We have been studying the biochemical control mechanisms in these cells in vitro. We report that enterochromaffin cells in the rabbit duodenum appear to contain muscarinic receptors which, when activated, lead to serotonin secretion on the vascular side of the mucosa.

Duodenal tissue was obtained from New Zealand White rabbits and the longitudinal and circular muscle layers were removed. The pieces of mucosa were mounted in modified Ussing chambers so that the serosal and mucosal surfaces could be incubated with separate solutions of Ringer bicarbonate. Test solutions could then be added to either or both sides of the preparations so that the vectorial release of serotonin could be studied (*6*). Preliminary studies showed that the spontaneous release of serotonin to the serosal side decreased and became stable during the first 30 minutes of incubation (Fig. 1). The spontaneous release of serotonin to the mucosal side was initially higher and more variable but also stabilized at a low level after 30 minutes.

The solutions bathing both sides of the tissue were then removed and replaced with control or drug-containing test solutions. Replacing the bathing solutions with control solutions caused some increase in the release of serotonin to the mucosal side, but release to the serosal side was not altered (Fig. 1). Since most of the serotonin released into the mucosal bathing chamber was removable by low-speed centrifugation, it probably was attributable to exfoliated enterochromaffin cells. When carbachol ($>10^{-6}M$) was added to the serosal side there was a dramatic increase in the

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release of serotonin to that side (Fig. 1). Maximal effects were found at about $10^{-5}M$ carbachol, which caused an approximately 20-fold increase over basal concentrations. However, addition of carbachol to the serosal side produced no increase in the mucosal release of serotonin. In addition, carbachol ($10^{-5}M$) added to the mucosal side produced no increase in the release of serotonin to either side. The release of serotonin following the addition of carbachol to the serosal side was not sustained and approached baseline values after 30 minutes.

Prior addition of atropine, a muscarinic cholinergic antagonist, had no effect on the release of serosal or mucosal serotonin but completely blocked the stimulation obtained with $10^{-5}M$ carbachol (Fig. 2). In contrast, prior incubation with the nicotinic antagonist hexamethonium had no effect on spontaneous or carbachol-stimulated release (Fig. 2). Prior incubation with the neuronal blocking agent tetrodotoxin ($10^{-6}M$) also had no effect on spontaneous or carbachol-stimulated serotonin release ($N = 4$), indicating that the serotonin released by carbachol was not of neuronal origin and that carbachol did not act through an interneuron. Acetylcholine chloride ($10^{-5}M$) added to the serosal side in the presence of neostigmine bromide ($10^{-4}M$) had the same effect on the serosal release of serotonin, both in magnitude and duration, as carbachol ($N = 3$). Neostigmine itself had no effect on the serosal release of serotonin. The addition of epinephrine and isoproterenol ($10^{-5}M$), bombesin, substance P, and neurotensin ($10^{-6}M$), or vasoactive intestinal polypeptide ($10^{-7}M$) to the serosal bathing medium had no effect on serosal or mucosal release of serotonin. The negative results with norepinephrine and isoproterenol contrast with previous results showing β-adrenoreceptor–mediated release of serotonin from enterochromaffin cells in cats and rats (*5*).

These results indicate that duodenal enterochromaffin cells in rabbits possess on their basal surfaces muscarinic receptors which, when activated by cholinergic agonists, stimulate vectorial release of serotonin. Such muscarinic receptors presumably are postsynaptic to cholinergic neurons. Indeed, unmyelinated nerve profiles and typical boutons have been demonstrated close to the basement membrane of enterochromaffin cells (*3*). The majority of these boutons are filled with clear round vesicles that probably contain acetylcholine. The role of the serotonin released is another question. The amine could act locally to modulate intestinal secretion (*7*) or to alter transmission in mucosal sensory neurons involved in gut vasodilator (*8*) or peristaltic reflexes (*9*). Traditional endocrine actions of serotonin exerted over longer distances are also possible (*10*).

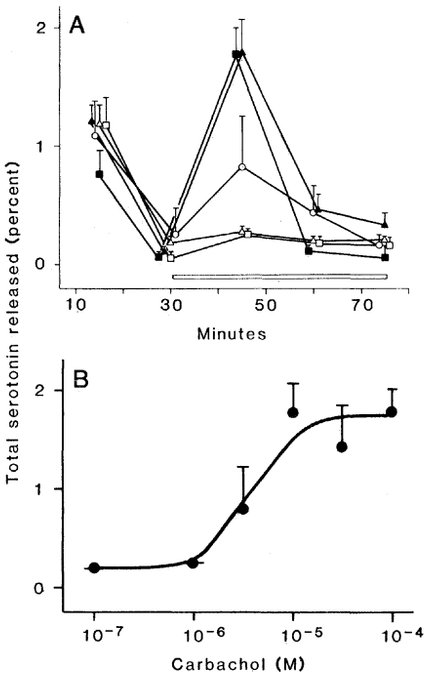

Fig. 1. (A) Release of serotonin to the serosal side following the addition of carbachol to the serosal side. Each value (mean ± standard error) is expressed as a percentage of the total serotonin measured for a given tissue sample. Symbols: (■) $10^{-4}M$ carbachol ($N = 4$); (▲) $10^{-5}M$ carbachol ($N = 8$); (○) $5 \times 10^{-6}M$ carbachol ($N = 3$); (□) $10^{-6}M$ carbachol ($N = 5$); and (△) Ringer bicarbonate ($N = 8$). Horizontal bar indicates the presence of carbachol on the serosal side. (B) Dose-response relation for the amount of serotonin released to the serosal side during the first 15 minutes of incubation with carbachol.

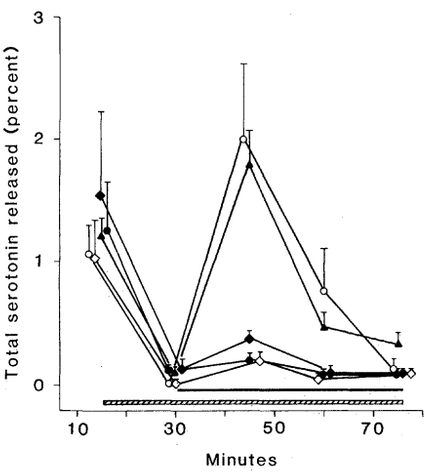

Fig. 2. Release of serotonin to the serosal side following the addition of various drugs to the serosal side. Each value (mean ± standard error) is expressed as a percentage of the total serotonin measured for a given tissue sample. Symbols: (▲) $10^{-5}M$ carbachol ($N = 8$); (●) $10^{-5}M$ carbachol plus $10^{-5}M$ atropine sulfate (Sigma) ($N = 4$); (◆) $10^{-5}M$ atropine sulfate ($N = 4$); (○) $10^{-5}M$ carbachol plus $10^{-5}M$ hexamethonium bromide (Sigma) ($N = 4$); and (◇) $10^{-5}M$ hexamethonium bromide ($N = 4$). The solid bar indicates the presence of carbachol and the hatched bar indicates the presence of atropine or hexamethonium.

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6. Removing the muscle layers effectively removed the myenteric and submucous nerve plexuses. Both sides of the tissue were incubated at 37°C with separate solutions of Ringer HCO_3^- buffer (2 ml per side) and continuously oxygenated. After 30 minutes the solutions were removed and replaced with test solution or buffer. Samples were removed every 15 minutes for serotonin determination. After a sample was taken, an equal volume of test solution or buffer (preheated and oxygenated) was added to replace the volume removed. Calculations of serotonin content were corrected for sample removal and volume replacement. The samples were immediately added to perchloric acid (final concentration, $0.4M$) containing an internal standard (5-hydroxy-*N*-methyl tryptamine oxalate, Sigma), which was used to correct for chromatographic variability. Serotonin was measured by using high-performance, strong-cation-exchange liquid chromatography with electrochemical detection [S. Sasa and C. L. Blank, *Anal. Chem.* **49**, 354 (1977)]. The samples were kept at 2°C in the dark before analysis and were centrifuged at $9000g$ for 5 minutes before being injected onto the column. After the last sample was taken the duodenal tissue that had been exposed to the incubation solutions was cut out and homogenized in $0.4M$ perchloric acid (which contained the internal standard) to extract the serotonin. The homogenate was centrifuged at $20{,}000g$ for 10 minutes and the supernatant was kept at 2°C in the dark until being injected onto the column. Standards were made by dissolving 5-hydroxytryptamine creatine sulfate complex (Sigma) and the internal standard in $0.4M$ perchloric acid.
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