Effect of VIP and PACAP on basal release of serotonin from isolated vascularly and luminally perfused rat duodenum

MINEKO FUJIMIYA,[1] HIROSHI YAMAMOTO,[1] AND ATSUKAZU KUWAHARA[2]
[1]*Department of Anatomy, Shiga University of Medical Science, Otsu, Shiga 520-21; and* [2]*Laboratory of Environmental Physiology, Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Shizuoka 422, Japan*

Fujimiya, Mineko, Hiroshi Yamamoto, and Atsukazu Kuwahara. Effect of VIP and PACAP on basal release of serotonin from isolated vascularly and luminally perfused rat duodenum. *Am. J. Physiol.* 275 (*Gastrointest. Liver Physiol.* 38): G731–G739, 1998.—The effect of vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide-38 (PACAP-38), and PACAP-27 on the release of serotonin (5-HT) into the intestinal lumen and the portal circulation was studied by using in vivo isolated vascularly and luminally perfused rat duodenum. 5-HT levels were determined by HPLC. VIP, PACAP-38, and PACAP-27 reduced the luminal release of 5-HT but did not affect the vascular release of 5-HT. The inhibitory effect caused by VIP, PACAP-38, and PACAP-27 was not affected by either atropine, hexamethonium, TTX, or TTX plus ACh, but it was completely antagonized by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine (L-NNA). The VIP receptor antagonist VIP-(10—28) blocked the effects of VIP, PACAP-38, and PACAP-27. These results suggest that VIP and PACAP exert a direct inhibitory effect on the luminal release of 5-HT from the enterochromaffin (EC) cells via a common receptor site on the EC cells and that this effect is mediated by NO but not by cholinergic pathways. A single injection of TTX, atropine, or hexamethonium reduced the luminal release of 5-HT, whereas a single injection of VIP-(10—28) stimulated the luminal release of 5-HT and this effect was antagonized by atropine, hexamethonium, or TTX. These results suggest that EC cells may receive the direct innervation of cholinergic neurons as well as VIP and/or PACAP neurons, with the former exerting a tonic stimulatory influence and the latter exerting a tonic inhibitory influence on 5-HT release into the intestinal lumen.

luminal release; isolated perfusion; vasoactive intestinal polypeptide; pituitary adenylate cyclase-activating peptide

THE MECHANISM THAT REGULATES the release of serotonin (5-HT) from the intestinal enterochromaffin (EC) cells has been widely investigated, using a variety of experimental models in different animals. Cholinergic and adrenergic neuronal mechanisms for regulation of 5-HT release have been most extensively examined in previous studies. In vitro experiments in isolated intestinal tissues of rats and cats have shown that a β-adrenergic mechanism mediated the release of 5-HT from EC cells measured by cytofluorometric methods (1, 29–31). In in vitro experiments in isolated sheets of rabbit intestine, the vectoral release of 5-HT into the mucosal or serosal side of the sheets has been shown to be mediated by a cholinergic mechanism (11, 12, 22, 23). A number of in vivo experiments have also been performed to investigate the mechanism for regulation of 5-HT release. In isolated vascularly perfused guinea pig intestine, muscarinic and nicotinic cholinergic mechanisms mediated the release of 5-HT (37, 38), and in isolated vascularly perfused dog intestine catecholamine stimulated the release of 5-HT (5). In isolated vascularly and luminally perfused cat intestine, a cholinergic mechanism stimulated the release of 5-HT into the intestinal lumen (2, 19, 20, 42); however, an adrenergic mechanism stimulated the release of 5-HT into the portal circulation (19, 20). Other mechanisms have also been examined. Intraluminal pressure caused the release of 5-HT into the lumen from isolated luminally and vascularly perfused rat duodenum (17) and from isolated loop of the guinea pig intestine (3, 4). A meal stimulated the luminal release of 5-HT (10), and hypertonic glucose stimulated the vascular release of 5-HT (7) from isolated perfused dog intestine. The effect of luminal acidification on 5-HT release has also been examined in both an in vivo study of rat intestine (34) or an in vitro experiment of an isolated sheet of rabbit intestine (12, 22, 23). In these experiments, low pH within the lumen stimulated the release of 5-HT into the intestinal lumen (22, 23, 34), and this effect was mediated by muscarinic cholinergic and β-adrenergic mechanisms (22, 23).

Although several previous studies have shown cholinergic and adrenergic neuronal involvement in 5-HT release, a relatively small number of studies have shown that nonadrenergic, noncholinergic (NANC) mechanisms regulate 5-HT release. In isolated vascularly perfused guinea pig intestine, direct inhibitory action of vasoactive intestinal polypeptide (VIP) on the release of 5-HT into the portal circulation has been shown; therefore, it is possible that this peptide is the NANC inhibitory neurotransmitter that regulates 5-HT release (32, 39). Other candidates for the NANC inhibitory neurotransmitter are ATP, which has been studied in isolated sheets of rabbit intestine (24, 32), and GABA, which has been studied in isolated vascularly perfused guinea pig intestine (40). In previous studies investigating the role of NANC mechanisms on the release of 5-HT, only the vascular release of 5-HT was examined. However, evidence has been shown that EC cells can release 5-HT into the intestinal lumen by demonstrating the intracellular immunoreactivities (17), and furthermore the release into the vasculature and the lumen is known to be mediated by independent mechanisms (20, 28). Therefore, the effects of the

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NANC inhibitory mechanism on the luminal release of 5-HT should be investigated in comparison to the vascular release of 5-HT.

In the present study, we aimed to examine the mechanism of action of VIP, pituitary adenylate cyclase activating peptide-38 (PACAP-38), and PACAP-27 on the release of 5-HT into the intestinal lumen as well as into the portal circulation. PACAP-38 and its shorter form PACAP-27 are newly isolated polypeptides and show structural homology with VIP (26, 27). Immunohistochemical localization of PACAP-27 (41) resembles that of VIP (6) in the gut; however, the role of this peptide on the release of 5-HT has not yet been examined. Furthermore, special attention has been paid to the relationship between VIP/PACAP and nitric oxide (NO), which has been demonstrated to be another NANC inhibitory neurotransmitter in the gastrointestinal tract (36).

MATERIALS AND METHODS

Male Wistar rats weighing 200–300 g were used. Care of animals was conducted in accordance with the *Guide to the Care and Use of Experimental Animals* (Shiga University of Medical Science). Animals were housed in a light-controlled room with free access to laboratory food and water but were fasted overnight (16–18 h) before the operation. Each animal was anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg Nembutal; Abbott Laboratories). The duodenum between the pylorus and Treitz's ligament was prepared for both vascular and intraluminal perfusion as described previously (15). Arterial perfusion was achieved through an aortic cannula with the tip lying adjacent to the celiac and superior mesenteric arteries, and effluent perfusate was collected through a portal vein cannula. All vasculature apart from that leading into the duodenal segment was cut between double ligatures. The stomach, jejunum, ileum, colon, pancreas, and spleen were removed. Luminal perfusion was performed through a cannula inserted into the pylorus; effluent perfusate was collected through a cannula placed into the duodenal lumen at the level of Treitz's ligament.

The vascular perfusate consisted of Krebs solution containing 3% dextran, 0.2% BSA (RIA grade; Sigma Chemical, St. Louis, MO), and 5 mM glucose. The perfusate was saturated with 95% O_2-5% CO_2 gas to maintain a pH of 7.4. We used 0.1 M PBS (pH 7.4) as a luminal perfusate. Both perfusates and the preparation were kept at 37°C throughout the experiment by a thermostatically controlled heating apparatus. The flow rates for vascular and luminal perfusion were maintained at 3 and 1 ml/min, respectively. After a 25-min equilibration period, both vascular and luminal effluents were collected at 3-min intervals for 33 min into ice-cold vials. Each vial contained 10 µl each of 57 mM ascorbic acid, 10 mM EDTA-2 Na, 1 M perchloric acid, and 51 mM pargyline hydrochloride in 1 ml of samples.

VIP (human and porcine), PACAP-38 (human), or PACAP-27 (human) (all obtained from the Peptide Institute, Osaka, Japan) was introduced into the vasculature via a side-arm infusion at a final concentration of 0.1 µM during perfusion *periods 5–7*. In some experiments, 1 µM TTX (Sankyo, Tokyo, Japan), 1 µM atropine sulfate (Sigma Chemical), 100 µM hexamethonium bromide (Sigma Chemical), 100 µM N^G-nitro-L-arginine (L-NNA; Peptide Institute), or 1 µM VIP-(10—28) (Sigma Chemical) was infused either singly during *periods 5–7* or combined with each of the above-mentioned peptides during *periods 4–7*. In some experiments, 1 µM TTX plus 1 µM ACh or only 1 µM ACh was infused combined with 0.1 µM VIP, 0.1 µM PACAP-38, or 0.1 µM PACAP-27; TTX was infused during *periods 4–8*, ACh was infused during *periods 5–8*, and each peptide was infused during *periods 6–8*. Furthermore, 10 mM L-arginine (Nacalai Tesque, Kyoto, Japan) plus 100 µM L-NNA were infused combined with 0.1 µM of VIP. L-Arginine was infused during *periods 1–8*, L-NNA was infused during *periods 4–8*, and VIP was infused during *periods 5–8*. In other experiments, 1 µM sodium nitroprusside (SNP) was infused singly during *periods 5–7* or combined with 1 µM TTX, which was infused during *periods 4–7*. Finally, 1 µM atropine, 100 µM hexamethonium, or 1 µM TTX was infused combined with 1 µM of VIP-(10—28). Atropine, hexamethonium, and TTX were infused during *periods 4–7*, and VIP-(10—28) was infused during *periods 5–7*.

We determined 5-HT levels by HPLC. Vascular effluents were filtrated with Ultrafree-MC (30,000 NMWL, Nihon Millipore, Yonezawa, Japan) by centrifuging for 30 min at 10,000 rpm at 4°C. Luminal effluents were filtrated manually with a 0.22-µm pore disk filter (Millex-GV, Nihon Millipore). We injected 100-µl aliquots of filtrates into HPLC and measured the 5-HT content (14). Results were expressed as means ± SE (in ng/min in each fraction).

Statistical analysis of data was performed using single-factor ANOVA for repeated measures followed by Scheffe's F test. A paired t-test (two-tail) was used to compare the values of mean basal release (during *periods 1–3* or *1–4*) and mean 5-HT release during periods as indicated in the results. In both cases, $P < 0.05$ was considered statistically significant.

RESULTS

The basal release of 5-HT into the intestinal lumen as well as into the vasculature was well maintained throughout the 33-min perfusion period. Basal 5-HT release into the lumen in 32 different experiments was 5.38 ± 0.24 ng/min (range 4.02–7.73 ng/min) and that into the vasculature was 2.28 ± 0.12 ng/min (range 1.52–3.81 ng/min).

When 1 µM TTX was infused, the release of 5-HT into the lumen was decreased after 3 min of exposure to TTX and stayed at a significantly lower level during perfusion *periods 6–11* (Fig. 1*A*). The mean level of luminal 5-HT release during *periods 6–11* (2.27 ± 0.23 ng/min, $n = 3$) was significantly lower than the mean basal release of 5-HT into the lumen (4.02 ± 0.19 ng/min), whereas the basal release of 5-HT into the vasculature was not affected by TTX. When 1 µM atropine was infused, the release of 5-HT into the lumen was decreased after 3 min of exposure to atropine. This suppression continued during perfusion *periods 6–11*, as shown in Fig. 1*B*. The mean level of luminal 5-HT release during *periods 6–11* (2.41 ± 0.68 ng/min, $n = 3$) was significantly lower compared with the mean basal release of 5-HT into the lumen (7.21 ± 0.76 ng/min). The basal release of 5-HT into the vasculature was not affected by atropine. Hexamethonium at a concentration of 1 µM did not affect either luminal or vascular release of 5-HT (data not shown). However, 100 µM hexamethonium strongly inhibited luminal release of 5-HT but did not affect vascular release of 5-HT, as shown in Fig. 1*C*. The mean basal release of 5-HT into the lumen as well as into the vasculature was not changed from the mean level of luminal or vascular

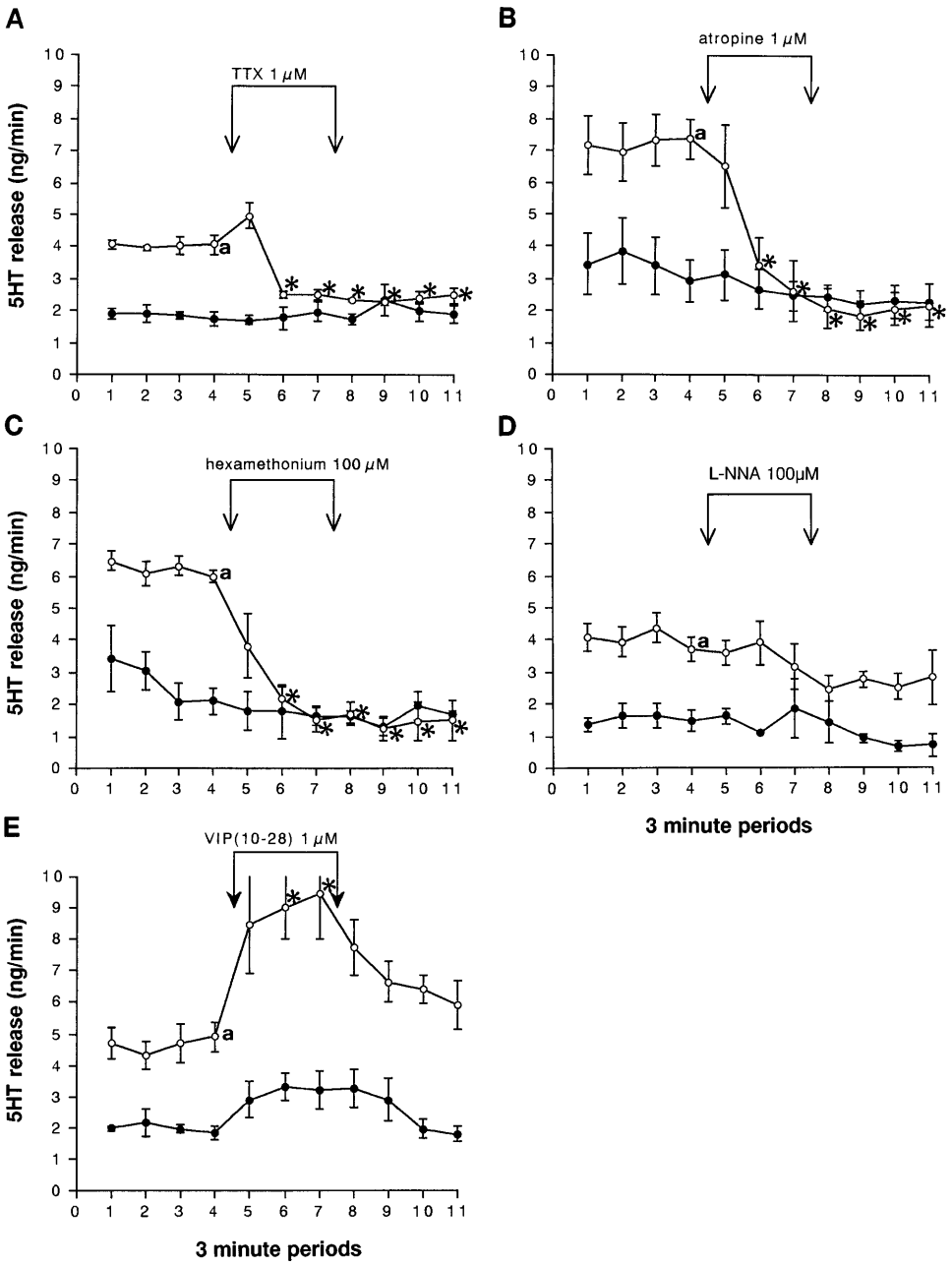

Fig. 1. Effect of TTX (*A*), atropine (*B*), hexamethonium (*C*), N^G-nitro-L-arginine (L-NNA) (*D*), and vasoactive intestinal polypeptide (VIP) receptor antagonist VIP-(10—28) (*E*) on luminal (○) and vascular (●) release of serotonin (5-HT) from isolated perfused rat duodenum. Values represent means ± SE of 3-min samples. *A–C*: $n = 3$. *D* and *E*: $n = 4$. Luminal release of 5-HT is significantly (*$P < 0.05$) decreased (*A*, *B*, *C*) and increased (*E*) compared with period before drug infusion (a).

release of 5-HT during infusion of 100 μM L-NNA (Fig. 1*D*). When 1 μM VIP-(10—28) was infused (Fig. 1*E*), the mean basal release of 5-HT into the lumen (4.65 ± 0.46 ng/min, $n = 4$) was significantly increased during perfusion *periods 6–7* (8.60 ± 1.05 ng/min, 213.96 ± 44.69% of basal release, see Fig. 6). However, the basal release of 5-HT into the vasculature was not affected by VIP-(10—28) (Fig. 1*E*).

When VIP was infused at a concentration of 0.1 μM, the basal release of 5-HT into the lumen (4.87 ± 0.61 ng/min, $n = 9$) was significantly decreased during perfusion *periods 7–8* (2.21 ± 0.32 ng/min, 51.46 ± 8.90% of basal release; Fig. 2). The vascular release of 5-HT was not affected by VIP (Fig. 2*A*). As shown in Fig. 1, luminal release of 5-HT was influenced by a cholinergic mechanism. Therefore, to determine whether the decrease in luminal 5-HT release caused by VIP was mediated by a cholinergic mechanism, we tested the effect of atropine (1 μM) as well as of hexamethonium (100 μM) on the VIP (0.1 μM)-induced decrease of 5-HT by introducing them 3 min before VIP administration. The basal release of 5-HT into the lumen before atropine infusion (4.74 ± 0.69 ng/min, $n = 3$) became significantly lower during *periods 7–10* (1.86 ± 0.12 ng/min, 40.26 ± 3.92% of basal release; Fig. 2*B*). Likewise, the basal release of 5-HT into the lumen before hexamethonium infusion (6.20 ± 0.72 ng/min, $n = 3$) was significantly decreased during *periods 6–8* (2.11 ± 0.60 ng/min, 35.58 ± 11.3% of basal release; Fig. 2*B*). There was no further reduction in the VIP response in the presence of either atropine or hexamethonium. A similar phenomenon was observed when TTX

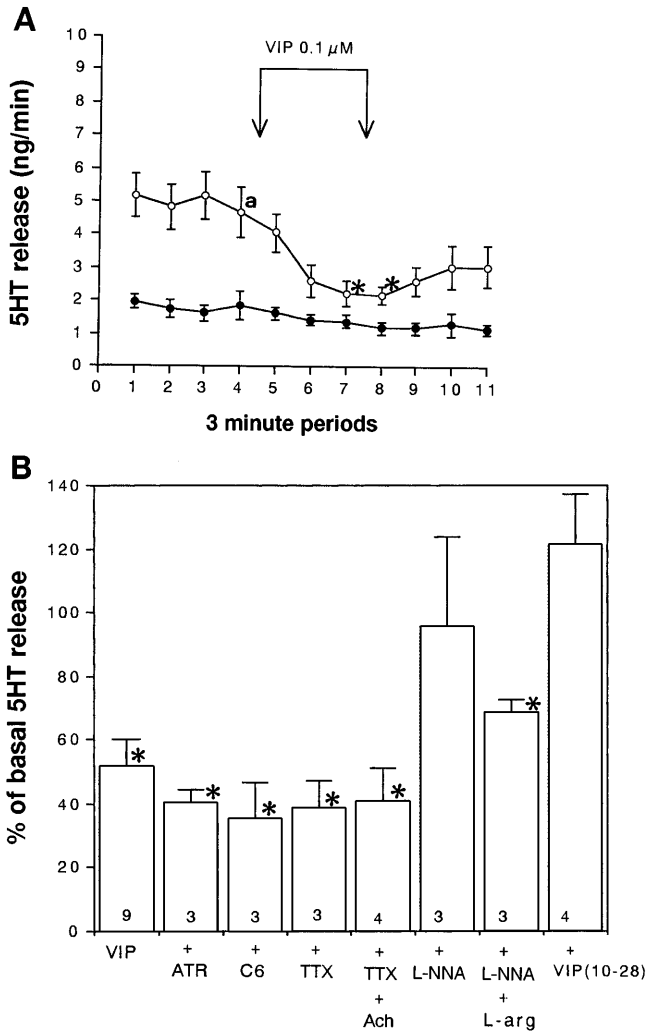

Fig. 2. *A*: effect of VIP ($n = 9$) on luminal (○) and vascular (●) release of 5-HT from isolated perfused rat duodenum. Values represent means ± SE of 3-min samples. Luminal release of 5-HT is significantly (*$P < 0.05$) decreased compared with period before drug infusion (a). *B*: effect of 0.1 μM VIP on luminal release of 5-HT, infused singly or combined with 1 μM atropine (Atr), 100 μM hexamethonium (C6), 1 μM TTX, 1 μM TTX + 1 μM ACh, 100 μM L-NNA, 100 μM L-NNA + 10 mM L-arginine (L-arg), or 1 μM VIP-(10—28). %Basal release is calculated as mean 5-HT release during periods described in RESULTS/mean basal 5-HT release × 100. Values represent means ± SE; *n* is shown at base of bars. *$P < 0.05$ vs. basal release.

(1 μM) was administered with VIP (0.1 μM). The basal release of 5-HT into the lumen (5.24 ± 0.41 mg/min, $n = 3$) became significantly lower during *periods 6–11* (2.03 ± 0.41 ng/min, 39.01 ± 8.37% of basal release; Fig. 2*B*). When a lower concentration of VIP (0.01 μM) was infused, basal release of 5-HT into the lumen (4.78 ± 0.58 ng/min, $n = 3$) became lower during *periods 7–8* (3.15 ± 0.43 ng/min, 67.01 ± 6.23% of basal release). Infusion of VIP (0.01 μM) with TTX (1 μM) inhibited luminal 5-HT release (38.72 ± 7.86% of basal release) more so than did VIP (0.01 μM) alone. In other experiments, when both TTX (1 μM) and ACh (1 μM) were infused with 0.1 μM VIP, the basal release of 5-HT into the lumen (6.76 ± 0.79 ng/min, $n = 4$) was significantly lower during perfusion *periods 7–11* (2.55 ± 0.36 ng/min, 41.04 ± 10.13% of basal release; Fig. 2*B*). In comparison, when ACh (1 μM) plus VIP (0.1 μM) were infused, ACh stimulated the basal release of 5-HT (124.3 ± 2.8% of basal release); however, the percent inhibition induced by VIP in the presence of ACh (42.9 ± 8.2% of basal release) did not change from that induced by TTX (1 μM) plus ACh (1 μM) plus VIP (0.1 μM). When the NO synthase (NOS) inhibitor L-NNA (100 μM) was introduced with VIP, the basal release of 5-HT into the lumen (4.63 ± 1.02 ng/min, $n = 3$) was not changed from the mean levels of luminal 5-HT release during *periods 5–7* (3.86 ± 0.34 ng/min, 95.44 ± 28.11% of basal release; Fig. 2*B*). When L-arginine (10 mM) and L-NNA (100 μM) were infused concomitantly with VIP, the basal release of 5-HT into the lumen (6.23 ± 0.67 ng/min, $n = 3$) became significantly lower during *periods 5–7* (4.26 ± 0.47 ng/min, 68.62 ± 4.00% of basal release; Fig. 2*B*). When 1 μM VIP-(10—28) was introduced with VIP, the basal release of 5-HT into the lumen (6.03 ± 0.18 ng/min, $n = 4$) was not changed from the mean level of luminal 5-HT release during *periods 5–7* (7.26 ± 0.82 ng/min, 121.23 ± 15.73% of basal release; Fig. 2*B*).

When 0.1 μM PACAP-38 was infused during perfusion *periods 5–7*, the basal release of 5-HT into the lumen (4.53 ± 0.28 ng/min, $n = 4$) was significantly decreased during perfusion *periods 7–9* (2.20 ± 0.23 ng/min, 48.42 ± 3.93% of basal release; Fig. 3), whereas the release of 5-HT into the vasculature was not affected by PACAP-38 (Fig. 3*A*). The effect of atropine (1 μM), hexamethonium (100 μM), TTX (1 μM), TTX plus ACh (1 μM), L-NNA (100 μM), or VIP-(10—28) (1 μM) on the inhibitory effect of PACAP-38 on luminal 5-HT release was examined. The basal release of 5-HT into the lumen before atropine infusion (4.25 ± 0.47 ng/min, $n = 3$) became significantly lower during *periods 6–11* (0.97 ± 0.15 ng/min, 22.52 ± 1.28% of basal release; Fig. 3*B*). The basal release of 5-HT into the lumen before hexamethonium infusion (6.78 ± 1.33 ng/min, $n = 4$) was decreased significantly during *periods 7–9* (1.52 ± 0.16 ng/min, 27.53 ± 9.45% of basal release; Fig. 3*B*). The basal release of 5-HT into the lumen before TTX infusion (4.74 ± 0.36 ng/min, $n = 3$) was lowered significantly during *periods 7–11* (1.55 ± 0.26 ng/min, 32.57 ± 4.55% of basal release; Fig. 3*B*). When TTX (1 μM) and ACh (1 μM) were infused concomitantly with 0.1 μM PACAP-38, the basal release of 5-HT into the lumen (7.73 ± 0.57 ng/min, $n = 3$) became significantly lower during perfusion *periods 7–11* (1.78 ± 0.61 ng/min, 25.28 ± 9.85% of basal release; Fig. 3*B*). When 100 μM L-NNA was introduced with PACAP-38, the basal release of 5-HT into the lumen (5.48 ± 0.42 ng/min, $n = 3$) was not changed from the mean level of luminal 5-HT release during *periods 5–7* (5.77 ± 0.75 ng/min, 106.21 ± 15.56% of basal release; Fig. 3*B*). When 1 μM VIP-(10—28) was introduced with PACAP-38, the basal release of 5-HT into the lumen (5.40 ± 0.22 ng/min, $n = 4$) was increased during *periods 5–7* (7.84 ± 0.80 ng/min, 146.51 ± 17.23% of basal release; Fig. 3*B*).

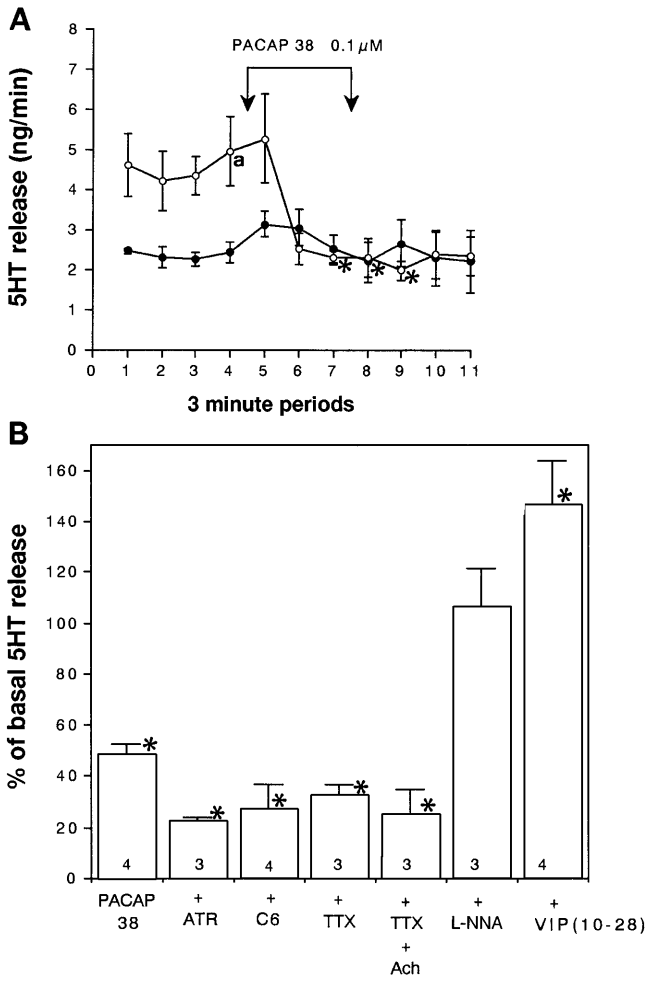

Fig. 3. *A*: effect of pituitary adenylate cyclase-activating peptide-38 (PACAP-38) ($n = 4$) on luminal (○) and vascular (●) release of 5-HT from isolated perfused rat duodenum. Values represent means ± SE of 3-min samples. Luminal release of 5-HT is significantly (*$P < 0.05$) decreased compared with period before drug infusion (a). *B*: effect of 0.1 μM PACAP-38 on luminal release of 5-HT, infused singly or combined with 1 μM atropine, 100 μM hexamethonium, 1 μM TTX, 1 μM TTX + 1 μM ACh, 100 μM L-NNA, or 1 μM VIP-(10—28). See Fig. 2 legend for explanation of %basal release. Values represents means ± SE; *n* is shown at base of bars. *$P < 0.05$ vs. basal release.

When PACAP-27 at a concentration of 0.1 μM was infused during perfusion *periods 5–7*, the basal release of 5-HT into the lumen (4.43 ± 0.80 ng/min, $n = 5$) was significantly decreased during *periods 7–9* (1.39 ± 0.27 ng/min, 31.50 ± 2.51% of basal release; Fig. 4). However, the vascular release of 5-HT was not affected by PACAP-27 (Fig. 4*A*). The effect of atropine (1 μM), hexamethonium (100 μM), TTX (1 μM), TTX plus ACh (1 μM), L-NNA (100 μM), or VIP-(10—28) (1 μM) on the inhibitory effect of PACAP-27 on luminal 5-HT release was examined. Again, the inhibitory response caused by PACAP-27 was not altered by administration of either atropine, hexamethonium, TTX, or TTX plus ACh; however, it was completely antagonized by administration of L-NNA and VIP-(10—28), as shown in Fig. 4*B*.

The effect of the NO donor SNP on the luminal and vascular release of 5-HT was examined (Fig. 5*A*). The mean basal release of 5-HT into the lumen (4.86 ± 0.33 ng/min, $n = 3$) was decreased significantly during *periods 5–6* (2.47 ± 0.15 ng/min, 50.97 ± 0.40% of basal release; Fig. 5*B*) and then rapidly returned to the basal level. The vascular release of 5-HT was not affected by SNP (Fig. 5*A*). This inhibitory effect of SNP on the luminal release of 5-HT was not antagonized by TTX, as shown in Fig. 5*B*.

To determine the relationship between cholinergic mechanisms and VIP neurons, we examined the effects of 1 μM VIP-(10—28) plus 1 μM atropine, 1 μM VIP-(10—28) plus 100 μM hexamethonium, and 1 μM VIP-(10—28) plus 1 μM TTX on 5-HT release. The stimulatory effect of VIP-(10—28) on the luminal release of 5-HT seen in Fig. 1*E* was completely blocked by the presence of either atropine, hexamethonium, or TTX (Fig. 6).

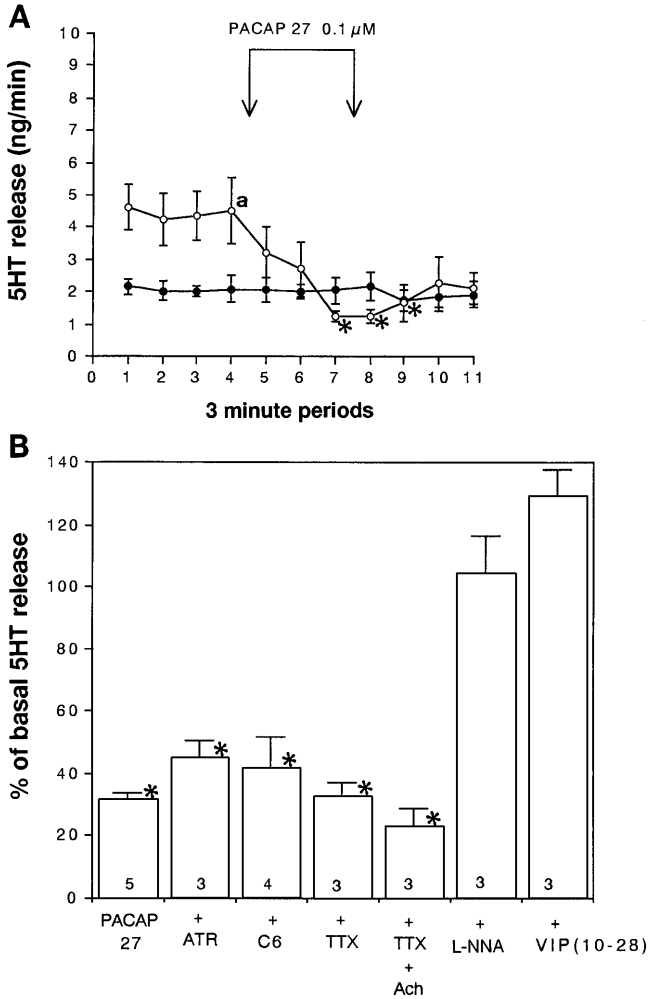

Fig. 4. *A*: effect of PACAP-27 ($n = 5$) on luminal (○) and vascular (●) release of 5-HT from isolated perfused rat duodenum. Values represent means ± SE of 3-min samples. Luminal release of 5-HT is significantly (*$P < 0.05$) decreased compared with period before drug infusion (a). *B*: effect of 0.1 μM PACAP-27 on luminal release of 5-HT, infused singly or combined with 1 μM atropine, 100 μM hexamethonium, 1 μM TTX, 1 μM TTX + 1 μM ACh, 100 μM L-NNA, or 1 μM VIP-(10—28). See Fig. 2 legend for explanation of %basal release. Values represent means ± SE; *n* is shown at base of bars. *$P < 0.05$ vs. basal release.

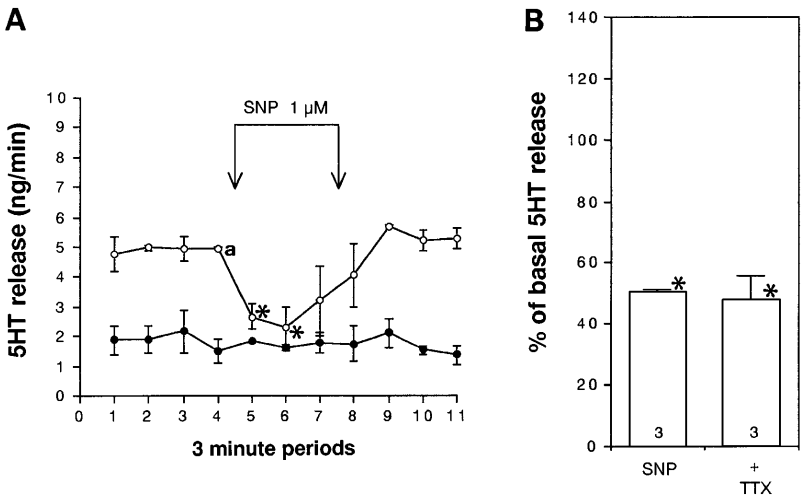

Fig. 5. *A*: effect of sodium nitroprusside (SNP) ($n = 3$) on luminal (○) and vascular (●) release of 5-HT from isolated perfused rat duodenum. Values represent means ± SE of 3-min samples. Luminal release of 5-HT is significantly (*$P < 0.05$) decreased compared with period before drug infusion (a). *B*: effect of 1 μM SNP on luminal release of 5-HT, infused singly or combined with 1 μM TTX. See Fig. 2 legend for explanation of %basal release. Values represent means ± SE; *n* is shown at base of bars. *$P < 0.05$ vs. basal release.

DISCUSSION

The present results show that a considerable amount of 5-HT was released from the isolated perfused rat duodenum into both the duodenal lumen and portal circulation and that the basal release of 5-HT into the lumen was always higher than that into the vasculature. Furthermore, the percent stimulation (or inhibition) of the basal release was much higher in the luminal release than in the vascular release in all experiments. Previously, the effects of cholinergic or noncholinergic mechanisms on the release of 5-HT have been studied in the guinea pig small intestine; however, only the vascular release of 5-HT was measured (32, 37–39, 40). These previous studies have shown that vascular release of 5-HT was much affected by several agents that showed little or no effect on the vascular 5-HT release in the present study. The discrepancy between the previous and present studies is not well understood. However, it may possibly be due to the difference in animal species or preparation. For example, in previous studies (13, 15, 16), the isolated loop of vascularly perfused guinea pig intestine was placed in the organ bath, but in the present study rat duodenum was treated with in vivo perfusion with intact extrinsic nerve supply into the intestinal segment. The release of 5-HT (2, 19, 42) or peptides such as somatostatin (15), gastrin (13), or peptide YY (16) into the intestinal lumen is widely accepted, and the mechanism for regulation is known to differ from that in vascular release. Recently, we have proposed morphological evidence for the luminal release of 5-HT from EC cells with demonstration of ultrastructural intracellular immunoreactivities (17). Results showed that EC cells without stimulation contained immunogold-labeled secretory granules in both apical and basal cytoplasm, while under stimulation of luminal release exocytotic granules were scarcely seen at the apical cell membrane, but immunogold label diffusely located over the apical cytoplasm and microvilli (17). In the present study, 5-HT detected in the lumen most likely originated from EC cells due to their localization in the epithelium as well as their higher 5-HT level compared with 5-HT-containing neurons in the gut. However, the contribution of 5-HT-containing nerves to luminal or at least to vascular release of 5-HT cannot be denied because a number of 5-HT-immunoreactive nerve fibers have been shown to distribute in the mucosal lamina propria or around the submucosal blood vessels in the rat duodenum (18).

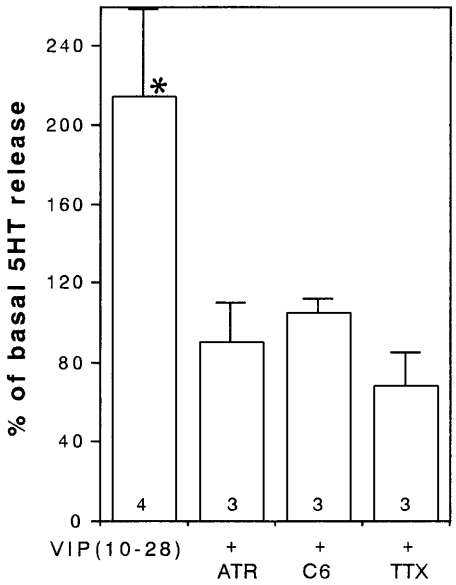

Fig. 6. Effect of 1 μM VIP-(10—28) on luminal release of 5-HT, infused singly or combined with 1 μM atropine, 100 μM hexamethonium, or 1 μM TTX. See Fig. 2 legend for explanation of %basal release. Values represent means ± SE; *n* is shown at base of bars. *$P < 0.05$ vs. basal release.

We examined the effects of the axonal blocker TTX, the cholinergic muscarinic blocker atropine, and the cholinergic nicotinic blocker hexamethonium on luminal and vascular release of 5-HT from the duodenum. The results show that TTX, atropine, and hexamethonium reduced the basal luminal release of 5-HT. Therefore, this suggests that the basal luminal release of 5-HT is tonically controlled by the neuronal pathways and both cholinergic muscarinic and nicotinic receptors were involved in this mechanism. The effect of cholinergic mechanisms on the luminal release of 5-HT was

examined previously in an in vivo experiment in dog jejunum (10) in which meal-induced stimulation of luminal release of 5-HT was blocked by intravenous injection of atropine. In in vivo experiments in cat jejunum (19), vagally mediated release of 5-HT into the lumen was blocked by atropine and hexamethonium administered intravenously. In an in vitro experiment (22) with isolated sheets of rabbit duodenum, acid-induced release of 5-HT into the luminal side of the sheet was blocked by both 1 μM atropine and 100 μM hexamethonium. Furthermore, from the findings in isolated vascularly perfused guinea pig ileum, the presence of muscarinic and nicotinic receptors on the EC cells has been indicated (37).

VIP inhibited the release of 5-HT into the lumen but did not affect the vascular release of 5-HT. This inhibitory effect of VIP was not affected by the presence of atropine, hexamethonium, or TTX. These results imply that VIP exerts a direct inhibitory effect on the luminal release of 5-HT from the EC cells and that other neuronal mechanisms, including cholinergic neurons, do not mediate this mechanism. This hypothesis was further confirmed by the finding that the luminal release of 5-HT was inhibited by VIP even in the presence of both TTX and ACh. VIP plus TTX had no greater effect than did VIP alone, except when a lower concentration of VIP was used. Similarly, VIP plus TTX plus ACh had no greater effect than did VIP plus ACh. These data suggest that the luminal release of 5-HT is neuronally mediated and that VIP and cholinergic neurons might be arranged in parallel but not in series. The effect of VIP on the release of 5-HT has previously been examined (39) in the isolated guinea pig small intestine, but only the vascular release of 5-HT was examined, in which VIP inhibited the vascular release of 5-HT and this effect was not affected by TTX. Although the direction of the release of 5-HT from the EC cells caused by VIP is different between the previous and present studies, it might be concluded that VIP exerts a direct inhibitory action on the luminal release of 5-HT from the EC cells. Endogenous VIP is involved in the neuronal cell bodies in both the myenteric and submucosal plexuses, and the number of VIP-positive neurons was much higher in the submucosal plexus than in the myenteric plexus (6). Furthermore, it has been reported that VIP-containing nerve fibers are located closely to the EC cells in the rat and guinea pig intestine (21). These morphological findings may suggest the direct action of VIP neurons on the EC cells.

The inhibitory effect of VIP on the luminal release of 5-HT was antagonized by the NOS inhibitor L-NNA. This effect of L-NNA was reversed by the pretreatment of the NOS substrate L-arginine. These data suggest that the inhibitory action of VIP on the luminal release of 5-HT from the EC cells is mediated by the NO pathway. Because a single injection of the NO donor SNP caused the reduction of luminal 5-HT release and this effect was not antagonized by TTX, it was suggested that NO exerts a direct inhibitory action on the EC cells and that no other neuronal mechanisms mediate this action. It is known that NO and VIP are functionally linked cotransmitters; for example, VIP release from myenteric neurons is regulated by NO production (25). Despite their functional cooperation, NOS-containing neurons were not always colocalized with VIP in both the myenteric and submucosal plexus (8, 9). The presence of NOS-containing neurons in the submucosal plexus has been demonstrated in the rat small intestine (8, 9). Therefore, these neurons are likely to mediate the inhibitory action of submucosal VIP neurons on the release of 5-HT from EC cells, although the detail of the interaction between NO and VIP neuronal cell bodies or nerve fibers in the submucosa or mucosa is not known at present.

Both PACAP-38 and PACAP-27 inhibited the release of 5-HT into the lumen but did not affect the vascular release of 5-HT. These inhibitory effects exerted by PACAP-38 or PACAP-27 on luminal 5-HT release were not changed by the presence of atropine, hexamethonium, or TTX. Furthermore, PACAP-38 and PACAP-27 reduced the luminal release of 5-HT even in the presence of both TTX and ACh, suggesting that PACAP-38 and PACAP-27 exert a direct inhibitory effect on the luminal release of 5-HT from the EC cells, but no other neuronal mechanisms, including cholinergic neurons, involved this mechanism. The effects observed in PACAP-38 and PACAP-27 were quite similar to those observed in VIP. PACAP-27-containing neurons have been shown to distribute in the myenteric and submucosal plexus, and the number of cell bodies was numerous in the submucosal plexus (41). Such distribution was quite similar to that of VIP-containing neurons in the intestine (6). Furthermore, some PACAP-27 neurons are known to colocalize with VIP neurons (41). The inhibitory effects seen in PACAP-38 and PACAP-27 were antagonized by L-NNA. This suggests that functional interaction exists both between VIP and NO and between PACAP and NO.

The VIP receptor antagonist VIP-(10—28) completely antagonized the inhibitory effect of VIP, PACAP-38, and PACAP-27 on the luminal release of 5-HT. The inhibitory effects of VIP, PACAP-38, and PACAP-27 were all abolished by the same concentration of VIP-(10—28). These data suggest that VIP and PACAP share a common receptor site on the EC cells of rat intestine. The common VIP/PACAP receptor, which has been characterized in human small intestine, has equal affinity to VIP, PACAP-38 and PACAP-27 (type II) and therefore is clearly differentiated from type I in the central nervous system (33, 35). The common VIP/PACAP receptor on the EC cells seems to possess the same characteristics as type II sites.

Finally, the results showed that the single injection of the VIP receptor antagonist VIP-(10—28) stimulated the luminal release of 5-HT, while the single injection of the NOS inhibitor L-NNA did not affect the basal release of 5-HT into the lumen. Furthermore, the stimulatory effect of VIP-(10—28) was completely antagonized by atropine, hexamethonium, or TTX. These results suggest that the luminal release of 5-HT may be caused by both tonic stimulatory influence from cholinergic mechanisms and tonic inhibitory influence from

VIP/PACAP pathways. Once the inhibitory effect is blocked by VIP-(10—28), the basal release of 5-HT seems to become abnormally high. NO-generating pathways, on the other hand, may not participate to sustain the basal release of 5-HT.

In conclusion, the present study provides evidence that VIP- and PACAP-generating pathways directly inhibit the luminal release of 5-HT from EC cells of rat duodenum and that they share a common receptor site, probably type II, on the EC cells. NO-generating pathways also directly inhibit luminal 5-HT release, and NO mediates the inhibitory action of VIP/PACAP. The results suggest that EC cells may receive the direct innervation of cholinergic neurons as well as VIP and/or PACAP neurons, with the former exerting a tonic stimulatory effect and the latter exerting a tonic inhibitory effect on the luminal release of 5-HT. Furthermore, VIP/PACAP are functionally linked with NO in this action.

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Address for reprint requests: M. Fujimiya, Dept. of Anatomy, Shiga Univ. of Medical Science, Seta, Otsu, Shiga 520-21, Japan.

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