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Adrenergic modulation of the release of 5-hydroxytryptamine from the vascularly perfused ileum of the guinea-pig

[1]K. Racké, H. Schwörer & H. Kilbinger

Department of Pharmacology, University of Mainz, Obere Zahlbacher Str. 67, D-6500 Mainz, F.R.G.

1 Isolated segments of the guinea-pig ileum were vascularly perfused and the release of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) into the portal venous effluent was determined by h.p.l.c. with electrochemical detection. Test substances were applied via the arterial perfusion medium.

2 Isoprenaline (0.1 μM) increased the outflow of 5-HT and 5-HIAA maximally by about 75% and this was antagonized by propranolol (0.1 μM). Forskolin (1–10 μM) increased the outflow of 5-HT by approximately 105% and that of 5-HIAA by approximately 55%. The phosphodiesterase inhibitor AH 21-132 (0.1–1 μM) increased the outflow of 5-HT and 5-HIAA by about 70%. Isoprenaline (1 nM) and AH 21-132 (10 nM), which alone had no effect, increased the outflow of 5-HT and 5-HIAA by 75%, when applied in combination.

3 Clonidine (1 μM) reduced the outflow of 5-HT by 45%, an effect blocked by tolazoline (1 μM), but not by prazosin (0.1 μM).

4 The effects of isoprenaline, forskolin and clonidine were also observed in the presence of tetrodotoxin (1 μM) demonstrating a direct modulation of 5-HT release from the enterochromaffin cells.

5 In conclusion, the release of 5-HT from enterochromaffin cells is facilitated by activation of β-adrenoceptors and inhibited via α_2-adrenoceptors. Enhancing intracellular cyclic AMP, by direct stimulation of adenylate cyclase with forskolin or by inhibition of phosphodiesterase, also facilitates the release of 5-HT. The β-adrenoceptor-mediated effect on 5-HT release appears to involve an increase in cyclic AMP, as the effect of isoprenaline was potentiated after inhibition of phosphodiesterase.

Introduction

Recent experiments from our laboratory (Schwörer *et al.*, 1987a) showed that the *in vitro* vascularly perfused ileum of the guinea-pig is very useful for studying the release of 5-hydroxytryptamine (5-HT) from enterochromaffin cells, the major source of intestinal 5-HT (Erspamer, 1966). Using this *in vitro* model we described the cholinergic mechanisms involved in the regulation of the release of 5-HT from the enterochromaffin cells (Schwörer *et al.*, 1987b).

There is evidence that adrenergic mechanisms may also participate in the regulation of intestinal 5-HT release. The intestinal mucosa is innervated by adrenergic nerve fibres which are often observed in close proximity to enterochromaffin cells (Lundberg *et al.*, 1978). Noradrenaline, adrenaline and isoprenaline decreased the mucosal 5-HT fluorescence of rat duodenal mucosa preparations incubated *in vitro*. This effect was antagonized by propranolol suggesting a β-adrenoceptor-mediated release of 5-HT (Pettersson *et al.*, 1978). Moreover, in the cat, the release of 5-HT into the portal circulation induced by vagal nerve stimulation *in vivo* was partially blocked by propranolol, suggesting that endogenous noradrenaline can also induce 5-HT release from the enterochromaffin cells via β-adrenoceptors (Pettersson *et al.*, 1979). In the dog vascularly perfused intestine, sympathomimetics or periarterial sympathetic nerve stimulation increased the outflow of 5-HT into the venous perfusate (Burks & Long, 1966).

In the present experiments, the effects of β- and α-adrenoceptor agonists and antagonists on the release of 5-HT from the vascularly perfused ileum of

[1] Author for correspondence.

the guinea-pig were studied. Since β-adrenoceptors are often linked to adenylate cyclase (see Lefkowitz *et al.*, 1983), the role of adenosine 3':5'-cyclic monophosphate (cyclic AMP) in the regulation of 5-HT release from the enterochromaffin cells was investigated in additional experiments with the phosphodiesterase inhibitor AH 21-132 (Markstein *et al.*, 1984) and forskolin, which directly stimulates adenylate cyclase (Seamon *et al.*, 1981). In addition, the effect of β-adrenoceptor activation was also studied in the presence of the phosphodiesterase inhibitor. The present experiments have been presented, in part, to the British Pharmacological Society (Schwörer *et al.*, 1988).

Methods

Preparation and perfusion of the ileal segments

Male guinea-pigs (300–500 g body weight) were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.) and artificially respired. The vascularly perfused ileum preparation was prepared as described (Holzer & Lembeck, 1979; Schwörer *et al.*, 1987a). The superior mesenteric artery was perfused with a modified Tyrode solution at 37°C at a constant rate of 1 ml min^{-1} by a peristaltic pump. The physiological salt solution contained (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, D-glucose 5.6, (+)-ascorbic acid 0.057, EDTA 0.03, and was equilibrated with 5% CO_2 + 95% O_2. The whole preparation, consisting of two or three loops, was put into a 200 ml organ bath which contained warmed (37°C) Tyrode solution bubbled with 95% O_2 + 5% CO_2. The oral end of the ileum was connected to a Marriotte bottle with two inlet tubes containing Tyrode solution (37°C). The aboral end was connected to an outflow cannula. During the first 60 min of the 90 min equilibration period peristalsis was induced every 15 min by raising the intraluminal pressure of the segment for 1 min by 500 Pa. After the equilibration period the intraluminal pressure remained zero. The Tyrode solution in the organ bath was renewed 30 min before the collection of the venous perfusate started.

The arterial perfusion pressure was measured by inserting a T-tube at the level where the arterial perfusion cannula was inserted into the superior mesenteric artery. This T-tube was filled with Tyrode solution and connected with an Optidynamic spinal fluid manometer (Mediplast). The arterial perfusion pressure varied between 1.3 and 1.6 kPa and was not significantly affected by perfusion with any of the test substances.

After the 90 min equilibration period the portal venous effluent was collected in 5 min fractions in acid-washed glass tubes, each containing 50 μl of 57 mM (+)-ascorbic acid, 50 μl of 10 mM EDTA and 65 μl of 1 M perchloric acid (suprapure). The test substances were added to the perfusion medium after 60 or 100 min. At the end of the collection period the tissue was freed from its mesentery and weighed. The weight of the segments was between 0.8 and 1.4 g.

Chemical analysis

5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured by h.p.l.c. with electrochemical detection (Schwörer *et al.*, 1987a). Briefly, the separation of 5-HT and its metabolite was achieved by a reverse phase column (length 250 mm, inner diameter 4.6 mm, prepacked with Shadon ODS-Hypersil, 5 μm) using a mobile phase of 0.1 M NaH_2PO_4 (adjusted to pH 3.0), containing octane sulphonic acid sodium salt (160 mg l^{-1}), sodium EDTA (0.3 mmol l^{-1}) and methanol (12%, v/v). The quantitation was achieved with an electrochemical detector (Chromatofield, ELCDe PA20 or Gynkothek, M 20) equipped with a glass carbon working electrode and an Ag/AgCl reference electrode. The potential was set at +0.74 V. Portions of 100 μl of the perfusate were injected directly onto the h.p.l.c.-column. The limit of detection was between 60 and 120 fmol for 5-HT and between 10 and 25 fmol for 5-HIAA per injection. The venous outflow of 5-HT and 5-HIAA is given in pmol g^{-1} 5 min^{-1}. The results are expressed as percentage of the mean outflow observed during the first two collection samples (90th–100th min of perfusion).

In the experiments in which the effect of isoprenaline was studied an additional peak with a retention time of about 15 min appeared in the chromatograms which could be identified as isoprenaline. This allowed the determination of the kinetics of this drug in the perfused ileum preparation (see results).

Statistics

Mean values of n experiments are given ± s.e.mean. The significance of difference between the two mean values was assessed by Student's t test. For comparison of one control with several experimental groups significance of differences was evaluated by the modified t test according to Bonferroni (see Wallenstein *et al.*, 1980).

Drugs

AH 21-132 (*cis*-6-(*p*-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo[c][1,6]-naphthyridine-*bis* (hydrogenmaleinate), Sandoz, Basel, Switzerland); forskolin dissolved in ethanol

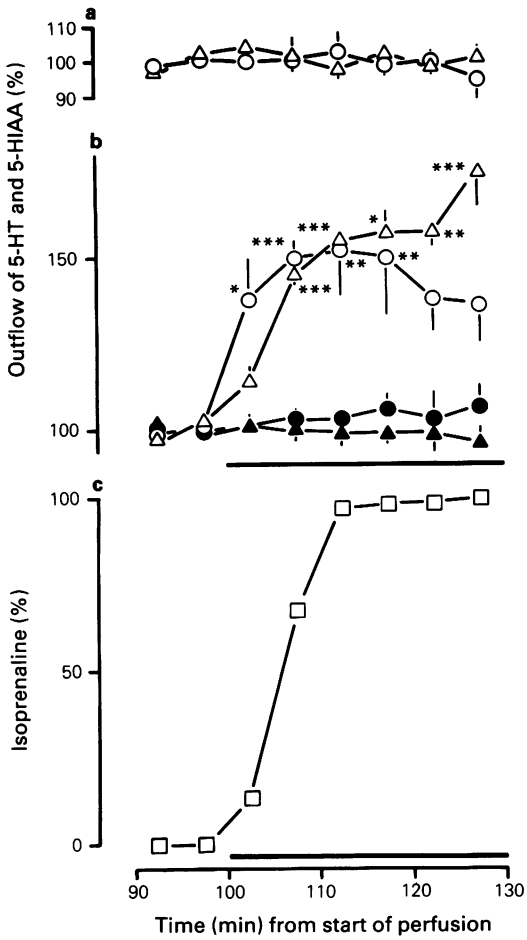

Figure 1 Effects of isoprenaline on the outflow of 5-hydroxytryptamine (5-HT; ○, ●) and 5-hydroxyindoleacetic acid (5-HIAA; △, ▲) from the isolated, vascularly perfused ileum of the guinea-pig. (a) A series of control experiments ($n = 7$). (b) Effect of 0.1 μM isoprenaline, in the absence (open symbols, $n = 4$) or presence (closed symbols, $n = 4$) of propranolol (0.1 μM). Isoprenaline was added to the perfusion medium as indicated by the horizontal bar; propranolol was added 40 min before isoprenaline. Ordinate scales (a and b), outflow of 5-HT and 5-HIAA expressed as % of the mean outflow from the 90th to 100th min of perfusion. During that time the outflow in absolute terms was 192 ± 11 pmol g^{-1} $5 min^{-1}$ for 5-HT and 216 ± 14 pmol g^{-1} $5 min^{-1}$ for 5-HIAA ($n = 15$). Symbols represent mean values of n experiments; vertical lines show s.e.mean. Significance of differences from the corresponding control value: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (c) Time course of the increase in concentration of isoprenaline in the venous effluent during perfusion with 0.1 μM isoprenaline (as indicated by the horizontal bar). Ordinate scale, venous isoprenaline concentration expressed as % of the venous equilibrium concentration (=sample 125–130 min). Mean values of 4 experiments are shown, s.e.mean smaller than the symbols. The venous equilibrium concentration of isoprenaline was 73.6 ± 4.5% of the arterial concentration.

(Calbiochem, Frankfurt, F.R.G.); clonidine HCl, (±)-isoprenaline HCl (C.H. Boehringer Sohn, Ingelheim am Rhein, F.R.G.); indomethacin (MSD Sharp & Dohme, München, F.R.G.); prazosin, dissolved in 50% ethanol, (Pfizer, Karlsruhe, F.R.G.); (±)-propranolol HCl (ICI-Pharma, Arzneimittelwerk Plankstadt, F.R.G.); tetrodotoxin, tolazoline HCl (Sigma, München, F.R.G.).

Results

After an equilibration period of 90 min the mean spontaneous outflow (determined between 90 and 100 min) was 176 ± 9 pmol g^{-1} $5 min^{-1}$ for 5-HT and 220 ± 10.1 pmol g^{-1} $5 min^{-1}$ for 5-HIAA ($n = 63$). In control experiments, the outflow of 5-HT and 5-HIAA did not change significantly during the following 30 min of perfusion (e.g. in Figure 1a). In agreement with previous observations (Schwörer *et al.*, 1987a,b), the outflow of 5-HT and its metabolite 5-HIAA was decreased, when tetrodotoxin (TTX, 1 μM) was added to the perfusion medium 30 min before the start of the collection period (to 96 ± 16 pmol g^{-1} $5 min^{-1}$ for 5-HT and 146 ± 27.2 pmol g^{-1} $5 min^{-1}$ for 5-HIAA, $n = 13$, each $P < 0.001$ vs the respective control value). However, during the 40 min collection period the outflow of 5-HT and 5-HIAA remained constant, when no additional drug was infused (not shown, $n = 4$).

Effects of isoprenaline

The β-adrenoceptor agonist isoprenaline (0.1 μM), added to the perfusion medium after 100 min of perfusion, increased the mean outflow of 5-HT by 50% (Figure 1). The maximum effect of isoprenaline was reached within 10 to 15 min and was maintained for 15 min. However, when the maximum effects of isoprenaline in each experiment were examined (either 10 or 15 min after the drug), an increase of 75% was calculated (Figure 2). With a delay of 5 min, the outflow of the metabolite 5-HIAA also increased. The highest value for 5-HIAA outflow (an increase of 75%) was observed in the last sample collected (Figure 1).

The rapid onset of the effect of isoprenaline became even more evident when the kinetics of the drug and the washout of 5-HT from the tissue were

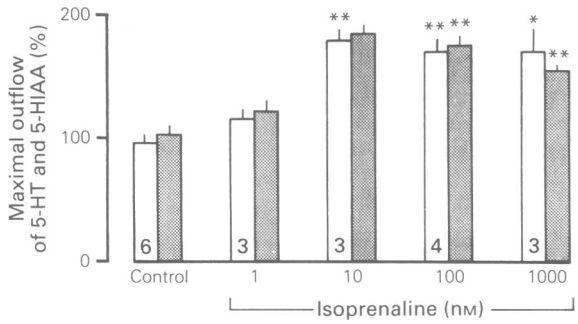

Figure 2 Concentration-dependent effects of isoprenaline on the outflow of 5-hydroxytryptamine (5-HT; open columns) and 5-hydroxyindoleacetic acid (5-HIAA; stippled columns) from the vascularly perfused ileum of the guinea-pig (for experimental protocol see Figure 1). Isoprenaline was added to the perfusion medium for 30 min from the 100th min onwards. Height of columns: outflow of 5-HT and 5-HIAA expressed as % of the mean outflow from the 90th to 100th min of perfusion. During that time the outflow in absolute terms was 177 ± 9 pmol g^{-1} $5\,min^{-1}$ for 5-HT and 219 ± 11 pmol g^{-1} $5\,min^{-1}$ for 5-HIAA ($n = 19$). Mean values of the maximal changes in each experiment are given with vertical lines indicating s.e.mean; the number of experiments is shown within the columns. Significance of differences from the controls: * $P < 0.05$; ** $P < 0.01$.

considered. As the h.p.l.c. with electrochemical detection also allowed the measurement of isoprenaline in the perfusion media, the time course of the appearance of isoprenaline in the venous effluent after onset of perfusion with the drug could be determined (Figure 1c). It should be pointed out that the kinetics of isoprenaline, as shown in Figure 1, reflect both parameters, invasion and washout of the drug, together. During the first 5 min of perfusion with 0.1 μM isoprenaline the increase in 5-HT outflow was nearly 80% of the maximum increase (Figure 1b), although the concentration of isoprenaline in the effluent reached only 13.4% of the equilibrium concentration, indicating that 0.1 μM isoprenaline is a supramaximal concentration. This is demonstrated in Figure 2 which shows a complete concentration-response histogram of isoprenaline. The maximum effects of isoprenaline on the outflow of 5-HT and 5-HIAA were observed at a concentration as low as 10 nM, but 1 nM isoprenaline had no effect.

The effect of 0.1 μM isoprenaline was prevented by 0.1 μM propranolol infused for 40 min before the agonist (Figure 1). In separate experiments in which propranolol (0.1 μM) was added to the medium after 100 min of perfusion, the outflow of 5-HT or 5-HIAA remained unaltered ($n = 4$, data not shown). In the presence of 1 μM TTX, isoprenaline (0.1 μM) increased the outflow of 5-HT by 59% (Table 1). Thus, the relative increase in 5-HT outflow in the presence of TTX is similar to that observed in the control experiments, but it should be noted that the outflow before the application of isoprenaline (in absolute terms) was about 45% lower in the presence of TTX compared to the control experiments (see above).

Table 1 Effects of tetrodotoxin on the modulatory effects of isoprenaline, forskolin and clonidine on the outflow of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) from the guinea-pig ileum

Outflow of 5-HT and 5-HIAA (%)

Absence of TTX:

	Iso	*For*	*Clo*
5-HT	170 ± 11	200 ± 21	55 ± 5
5-HIAA	175 ± 9	156 ± 9	74 ± 3
n	4	3	4

Presence of TTX:

	Iso	*For*	*Clo*
5-HT	159 ± 4	210 ± 54	65 ± 4
5-HIAA	184 ± 7	169 ± 42	67 ± 5
n	3	3	3

Isolated segments of the guinea-pig ileum were vascularly perfused as described in Figure 1 and the outflow of 5-HT and 5-HIAA was determined. Isoprenaline (Iso, 0.1 μM), forskolin (For, 1 μM) or clonidine (Clo, 1 μM) were intra-arterially infused after 100 min of perfusion. Tetrodotoxin (TTX, 1 μM) was present 40 min before the test drugs. The outflow of 5-HT and 5-HIAA is expressed as % of the mean outflow from the 90th–100th min of perfusion. During that time the outflow in absolute terms was 191 ± 15 pmol g^{-1} $5\,min^{-1}$ for 5-HT and 202 ± 12 pmol g^{-1} $5\,min^{-1}$ for 5-HIAA in the absence of TTX ($n = 11$) and 119 ± 11 pmol g^{-1} $5\,min^{-1}$ for 5-HT and 120 ± 13 pmol g^{-1} $5\,min^{-1}$ for 5-HIAA, in the presence of TTX ($n = 9$). Mean values of the maximal changes in each experiment are given $\pm$ s.e.mean with the number of experiments (n) indicated.

Effects of AH 21-132 and forskolin

The phosphodiesterase inhibitor AH 21-132 increased the outflow of 5-HT maximally by 75% and that of 5-HIAA by 60% (Figure 3). AH 21-132 at a concentration as low as 0.1 μM almost induced the maximum response.

When 10 nM AH 21-132, which alone had no effect (Figure 4b), was applied in combination with 1 nM isoprenaline which also had no significant effect alone (Figure 4a) the outflow of 5-HT was increased maximally by 75 ± 14% and that of 5-HIAA by 64 ± 16% (Figure 4c).

Forskolin, at concentrations of 1 μM and 10 μM,

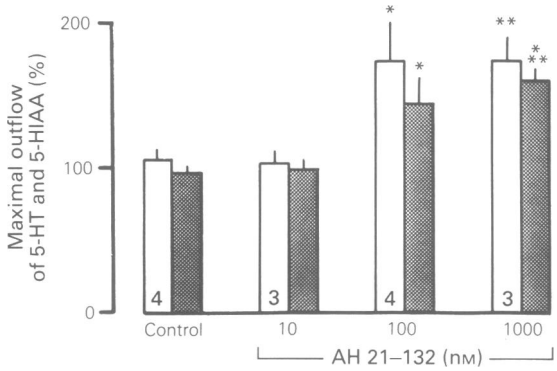

Figure 3 Concentration-dependent effects of AH 21-132 on the outflow of 5-hydroxytryptamine (5-HT; open columns) and 5-hydroxyindoleacetic acid (5-HIAA; stippled columns) from the vascularly perfused ileum of the guinea-pig (for experimental protocol see Figure 1). AH 21-132 was added to the perfusion medium for 30 min from the 100th min onwards. Height of columns: outflow of 5-HT and 5-HIAA expressed as % of the mean outflow from the 90th to 100th min of perfusion. During that time the outflow in absolute terms was 190 ± 15 pmol g^{-1} $5\,min^{-1}$ for 5-HT and 239 ± 19 pmol g^{-1} $5\,min^{-1}$ for 5-HIAA ($n = 14$). Mean values of the maximal changes in each experiment are given with vertical lines indicating s.e.mean; the number of experiments is shown within the columns. Significance of differences from the controls: * $P < 0.05$; ** $P < 0.01$.

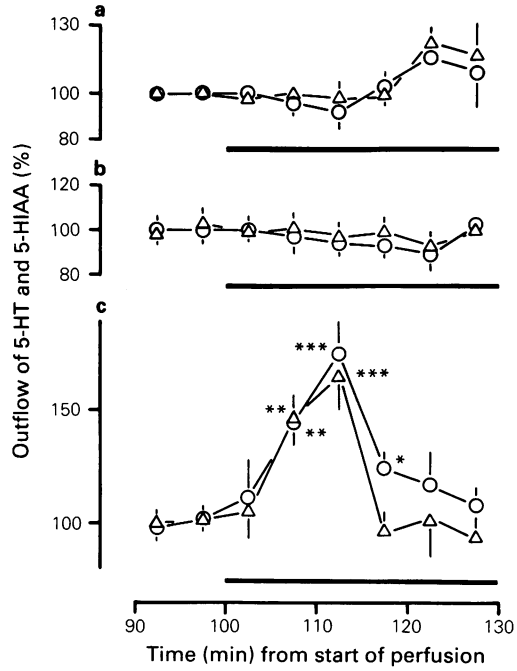

Figure 4 Effects of 1 nM isoprenaline alone (a), 10 nM AH 21-132 alone (b) or both in combination (c) on the outflow of 5-hydroxytryptamine (5.HT; ○) and 5-hydroxyindoleacetic acid (5-HIAA; △) from the vascularly perfused ileum of the guinea-pig (for experimental protocol see Figure 1). Isoprenaline and/or AH 21-132 were added to the perfusion medium as indicated by the horizontal bars. Ordinate scales: outflow of 5-HT and 5-HIAA expressed as % of the mean outflow from the 90th to 100th min of perfusion. During that time the outflow in absolute terms was 177 ± 15 pmol g^{-1} $5\,min^{-1}$ for 5-HT and 240 ± 16 pmol g^{-1} $5\,min^{-1}$ for 5-HIAA ($n = 10$). Given are mean values of 3–4 experiments; vertical lines indicate s.e.mean. Significance of differences from the corresponding control value: ** $P < 0.01$; *** $P < 0.001$.

increased the outflow of 5-HT by 107% and 101%, respectively. Enhancing the concentration of forskolin to 30 μM induced an only marginally larger increase (Figure 5). Forskolin (1, 10 and 30 μM) also increased the outflow of the metabolite 5-HIAA by 56%, 44% and 51%, respectively. At 0.1 μM forskolin did not significantly affect the outflow of 5-HT and 5-HIAA (Figure 5). Similar to the effects of isoprenaline, the maximal response to forskolin was observed within 10–15 min after addition of the drug to the perfusion medium (not shown). In the presence of 1 μM TTX, forskolin (1 μM) increased the outflow of 5-HT by 110% (Table 1). Since forskolin in low concentrations (1 μM) has only small direct effects on adenylate cyclase, but strongly augments receptor-mediated activation of adenylate cyclase (e.g. by β-adrenoceptor agonists or prostaglandins, Daly *et al.*, 1982), the effect of 1 μM forskolin was also studied in the presence of 0.1 μM propranolol or 1 μM indomethacin (each drug present 40 min before forskolin). The effect of forskolin remained unaltered, both in the presence of propranolol (the outflow of 5-HT increased by $94 \pm 14\%$ ($n = 3$, $P < 0.01$ vs controls) or indomethacin (the outflow of 5-HT increased by $79 \pm 25\%$, $n = 4$, $P < 0.01$ vs controls). Indomethacin alone (1 μM, added after 100 min of perfusion) had no effect on the outflow of 5-HT or 5-HIAA ($n = 3$, data not shown).

Effects of clonidine

The α_2-adrenoceptor agonist clonidine (1 μM) reduced the outflow of 5-HT by $45 \pm 5\%$ within 10 min of perfusion (Figure 6). During the following 20 min this effect partially disappeared despite the continuous presence of the drug. Clonidine also decreased the outflow of 5-HIAA, although less prominently (Figure 6). At a concentration of 0.1 μM, clonidine reduced the outflow of 5-HT maximally by $36 \pm 6\%$ ($n = 3$, $P < 0.001$ vs controls). Figure 6

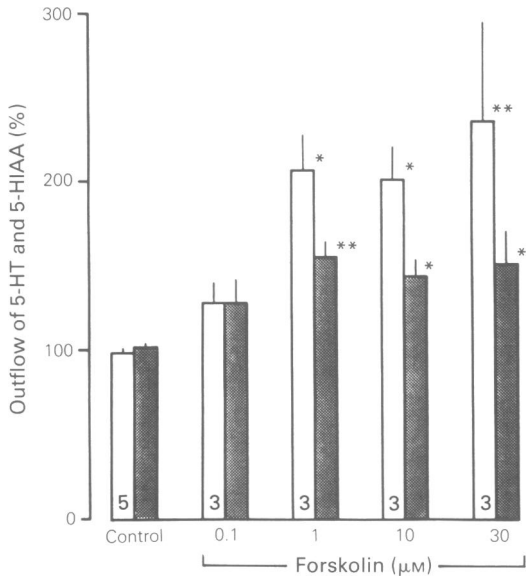

Figure 5 Concentration-dependent effects of forskolin on the outflow of 5-hydroxytryptamine (5-HT; open columns) and 5-hydroxyindoleacetic acid (5-HIAA; stippled columns) from the vascularly perfused ileum of the guinea-pig (for experimental protocol see Figure 1). Forskolin was added to the perfusion medium for 30 min from the 100th min onwards. In control experiments ethanol (2.3 mM, the maximum concentration applied with forskolin) was perfused from the 100th min onwards. Height of columns: outflow of 5-HT or 5-HIAA expressed as % of the mean outflow from the 90th to 100th min of perfusion. During that time the outflow in absolute terms was 177 ± 18 pmol g^{-1} $5 min^{-1}$ for 5-HT and 224 ± 19 pmol g^{-1} $5 min^{-1}$ for 5-HIAA ($n = 17$). Mean values of the maximal changes in each experiment are given with vertical lines indicating s.e.mean; the number of experiments is shown within the columns. Significance of differences from the controls: * $P < 0.05$; ** $P < 0.01$.

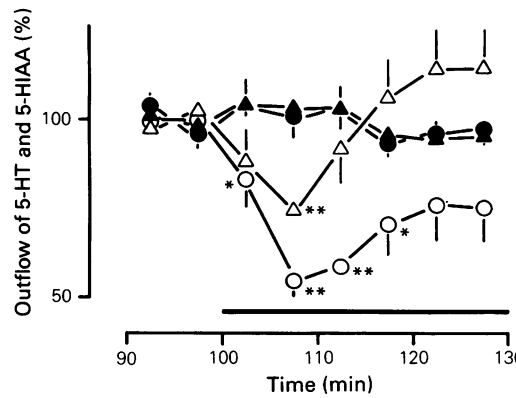

Figure 6 Effects of clonidine (1 μM) on the outflow of 5-hydroxytryptamine (5-HT; ○, ●) and 5-hydroxyindoleacetic acid (5-HIAA; △, ▲) from the isolated, vascularly perfused ileum of the guinea-pig in the absence (open symbols, $n = 4$) and presence (closed symbols, $n = 3$) of 1 μM tolazoline. Clonidine was added to the perfusion medium as indicated by the horizontal bar; tolazoline was already present 40 min before clonidine. In control experiments (=absence of drugs), the outflow of 5-HT and 5-HIAA did not change significantly during the collection period, ($n = 5$, not shown, see also Figure 1). Ordinate scale: outflow of 5-HT and 5-HIAA expressed as % of the mean outflow from the 90th to 100th min of perfusion. During that time the outflow in absolute terms was 169 ± 12 pmol g^{-1} $5 min^{-1}$ for 5-HT and 193 ± 15 pmol g^{-1} $5 min^{-1}$ for 5-HIAA ($n = 12$). Mean values of n experiments are shown; vertical lines indicate s.e.mean. Significance of differences from the corresponding control value: * $P < 0.05$; ** $P < 0.001$.

also shows that the effect of 1 μM clonidine was prevented by the α_2-adrenoceptor antagonist tolazoline (1 μM). On the other hand, the inhibitory effect of 1 μM clonidine remained unchanged in the presence of 0.1 μM prazosin (present 40 min before the agonist), the outflow of 5-HT decreased by $58 \pm 6\%$ and that of 5-HIAA by $45 \pm 14\%$ ($n = 3$, $P < 0.01$ vs controls). The affinity constant of prazosin for the α_1-adrenoceptor is in the order of 1 nM and that of tolazoline for the α_2-adrenoceptor about 180 nM (see Starke, 1981; Langer, 1981). In separate experiments, neither prazosin (0.1 μM) nor tolazoline (1 μM) alone (each drug added to the perfusion medium after 100 min of perfusion) altered the outflow of 5-HT or 5-HIAA (each $n = 3$, data not shown). The inhibitory effect of clonidine was also observed in the presence of 1 μM TTX (Table 1).

Discussion

The present results demonstrate that the release of 5-HT from the vascularly perfused guinea-pig ileum can be modulated via β- and α_2-adrenoceptors. As discussed in detail previously (Schwörer *et al.*, 1987a), the spontaneous outflow of 5-HT and its metabolite 5-HIAA into the portal circulation of the vascularly perfused ileum of the guinea-pig largely reflects calcium-dependent release of 5-HT, derived almost exclusively from the enterochromaffin cells. Part of the so called spontaneous 5-HT release from the present *in vitro* preparation is triggered by a spontaneous neuronal (cholinergic) stimulant input (Schwörer *et al.*, 1987b). In confirmation of these previous observations, the spontaneous outflow of 5-HT and 5-HIAA in the present experiments was also

reduced by TTX, which blocks voltage-dependent sodium channels (Narahashi *et al.*, 1964) and therefore prevents neuronal activity.

Isoprenaline increased the outflow of 5-HT and 5-HIAA and this response was antagonized by propranolol. As the effect of isoprenaline was also seen in the presence of TTX, it can be concluded that β-adrenoceptors located on enterochromaffin cells facilitate the release of 5-HT. Thus, these experiments confirm and extend the conclusion obtained from experiments in which the release of 5-HT from the enterochromaffin cells of rat duodenum was estimated indirectly by measuring changes in tissue 5-HT fluorescence (Pettersson *et al.*, 1978).

Stimulation of adenylate cyclase by forskolin or increasing the intracellular cyclic AMP levels by inhibition of phosphodiesterase by AH 21-132 also caused an increase in 5-HT release. β-Adrenoceptors are often linked to adenylate cyclase (see Lefkowitz *et al.*, 1983). The present results, showing that the effect of isoprenaline is potentiated by the phosphodiesterase inhibitor AH 21-132, support the conclusion that an activation of adenylate cyclase may be involved in β-adrenoceptor-mediated facilitation of 5-HT release from the enterochromaffin cells of the guinea-pig ileum. A role for cyclic AMP in the regulation of 5-HT release from enterochromaffin cells of rabbit duodenum was also demonstrated by Forsberg & Miller (1983). Strikingly, forskolin caused the maximum increase in 5-HT release at a concentration of 1 μM. Since at this concentration forskolin has only marginal, direct stimulative effects on adenylate cyclase, but strongly augments receptor-mediated effects on adenylate cyclase (Daly *et al.*, 1982), it may be concluded that the facilitation of 5-HT release by forskolin is caused by an augmentation of an endogenous stimulator of adenylate cyclase. However, the potentiation of the effect of a subthreshold-concentration of endogenous catecholamines (the concentrations of noradrenaline, adrenaline and dopamine in the venous effluent of the present preparation are between 100 and 200 pM for each catecholamine, unpublished observations) via β-adrenoceptor activation can be excluded, as the effect of 1 μM forskolin was also observed in the presence of propranolol. The potentiation by forskolin of the stimulative effects of other, spontaneously released neurotransmitter substances may also be excluded under the present *in vitro* conditions, because forskolin (1 μM) also increased the release of 5-HT in the presence of TTX. Forskolin, in addition, augments the stimulation of adenylate cyclase by prostaglandins (Daly *et al.*, 1982). Indomethacin did not affect the stimulant effect of 1 μM forskolin, excluding an augmentation of the effect of endogenous prostaglandins. Thus, the stimulation of 5-HT release by 1 μM forskolin may involve other unknown local stimulators of adenylate cyclase, but it is also possible that in enterochromaffin cells only very small changes in intracellular cyclic AMP have substantial effects on 5-HT release.

The same mechanisms which might be responsible for the high sensitivity of forskolin may also cause the high sensitivity of the phosphodiesterase inhibitor AH 21-132. In addition, there is evidence for the existence of several subclasses of cyclic AMP-specific phosphodiesterases (see Weishaar *et al.*, 1987) and AH 21-132 could have a particularly high affinity for the one present in the enterochromaffin cells.

Clonidine, a selective α_2-adrenoceptor agonist (see Starke, 1981; Langer, 1981), inhibited the release of 5-HT from the enterochromaffin cells. This effect was prevented by tolazoline, a selective α_2-adrenoceptor antagonist, but not by prazosin, a selective α_1-adrenoceptor antagonist (see Starke, 1981; Langer, 1981). The inhibitory effect of clonidine was observed in the presence of TTX suggesting that the α_2-adrenoceptor-mediated inhibition of 5-HT release is, at least partially, a direct effect on the enterochromaffin cells. Under similar experimental conditions the inhibition of 5-HT release via muscarinic receptor activation seemed to be mediated completely by the release of an inhibitory neurotransmitter (Schwörer *et al.*, 1987b). Although α_2-adrenoceptors are often linked negatively to adenylate cyclase (Lefkowitz *et al.*, 1983), the present experiments do not allow any conclusion to be drawn about the biochemical mechanisms involved in the inhibition of 5-HT release via α_2-adrenoceptor activation.

It should be mentioned that the effects of isoprenaline, forskolin or clonidine were expressed as % change in the outflow of 5-HT or 5-HIAA in comparison to the pre-drug outflow. These 'relative' effects of all three drugs were similar in the presence and absence of TTX, although the pre-drug outflow of 5-HT and 5-HIAA was about 45% lower in the presence of TTX. One explanation for this observation may be that only the efficiency of 'secretory active' cells is enhanced by isoprenaline and forskolin or reduced by clonidine. Other modulatory inputs may regulate the number of 'active' cells. For example, a neuronal, nicotinic stimulative input which has been demonstrated previously (Schwörer *et al.*, 1987b), could activate 'silent cells', the secretory efficiency of which could then be modulated by the above discussed mechanisms. However, beside their direct effects on the enterochromaffin cells, isoprenaline, forskolin and clonidine may also modulate the neuronal input to these cells.

Finally, some possible functional consequences of the present results should be mentioned. An enhanced secretion of 5-HT appears to be involved in the pathogenesis of diarrhoea caused by cholera toxin (Beubler *et al.*, 1987) and carcinoid syndrome

(Anderson *et al.*, 1987). The present observation that activation of adenylate cyclase by forskolin increases intestinal 5-HT release is in agreement with the observation that cholera toxin, which also activates adenylate cyclase (Sharp & Hynie, 1971; Kimberg *et al.*, 1971), stimulates intestinal 5-HT release (Nilsson *et al.*, 1983; Beubler *et al.*, 1987). Furthermore, α_2-adrenoceptor agonists have antidiarrhoeal properties (see Dharmsathaphorn, 1986) and also inhibit the cholera toxin-induced intestinal fluid accumulation (Nakaki *et al.*, 1982). It may be speculated that inhibition of 5-HT release by α_2-adrenoceptor activation, as demonstrated in the present experiments, may contribute to the antidiarrhoeal action of α_2-adrenoceptor agonists.

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