Pharmacological Characterization of Presynaptic α -Adrenoceptors in the Modulation of the 5-Hydroxytryptamine Release from Vascular Adrenergic Nerves in the Rat

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Abstract—The role of presynaptic α -adrenoceptors in modulation of the 5-hydroxytryptamine (5-HT) release from vascular adrenergic nerves was investigated in the perfused mesenteric vascular bed of the rat. After treatment with 5-HT (10 μ M) for 15 min, the vasoconstrictor response to periaarterial nerve stimulation (PNS, 4 to 16 Hz, 2 msec in duration for 30 sec) was greatly potentiated without significantly affecting the pressor response to exogenously administered noradrenaline (0.5 nmol). The potentiating effect was more pronounced at low frequencies of PNS (4 and 8 Hz). The potentiation of the pressor response to PNS after 5-HT treatment did not occur in the presence of LY53857 (0.01 μ M), a selective 5-HT₂ receptor antagonist. The enhanced pressor response to PNS seen after 5-HT treatment was further exaggerated in the presence of clonidine (0.1 and 1 μ M), a preferential α_2 -adrenoceptor agonist, while methoxamine (1 and 10 μ M), a selective α_1 -adrenoceptor agonist, did not affect the enhanced PNS response. This effect of clonidine was more pronounced in low frequencies of PNS (4 and 8 Hz) and was abolished by LY53857 (0.01 μ M). In the perfused mesenteric vascular bed labelled with [³H]-5-HT, PNS (8 Hz) evoked an increase of tritium efflux in the perfusate. The PNS-evoked tritium efflux was facilitated by yohimbine (0.1 to 1 μ M), an α_2 -adrenoceptor antagonist, and prazosin, a selective α_1 -adrenoceptor antagonist, at a high concentration (1 μ M), while LY53857 (0.01 to 0.1 μ M) and a low concentration of prazosin (0.1 μ M) had no effect on the tritium efflux. Clonidine (0.01 to 1 μ M) produced a dose-dependent increase of PNS-evoked tritium efflux, while methoxamine (0.1 to 10 μ M) was without effect. The monoamine uptake inhibitor, cocaine (10 μ M) produced a significant inhibition of the PNS-evoked tritium efflux. The effects of clonidine and cocaine on the PNS-evoked tritium efflux were antagonized by yohimbine (1 μ M). These results suggest that the release of 5-HT from adrenergic nerve endings by PNS is modulated by presynaptic α_2 -adrenoceptors.

There is increasing evidence that 5-hydroxytryptamine (5-HT) accumulates into the adrenergic nerve endings of various tissues including guinea-pig vas deferens (1), guinea-pig intestine (2), rat pineal gland (3), canine saphenous vein and cerebral artery (4), and rat mesenteric artery (5). The 5-HT taken up and accumulated into vascular adrenergic nerves is released by nerve stimulation in a calcium-dependent manner (4, 5). Additionally, the released 5-HT from

adrenergic nerve endings produces a vasoconstriction, which is mediated by postsynaptic 5-HT₂ receptors (5).

It is generally accepted that presynaptic α -adrenoceptors, especially the α_2 -subtype, on sympathetic, adrenergic nerve endings can modulate the neurotransmitter (noradrenaline, NA) release (6, 7). In an earlier report (5), we demonstrated that the release of 5-HT from vascular adrenergic nerves is carried out by the calcium-dependent exocytotic process like

NA, suggesting that the 5-HT release may be modulated by presynaptic α -adrenoceptors. However, it is not yet clear whether the modulation is mediated via α_1 - or α_2 -adrenoceptors.

The present study was designed, therefore, to examine which receptor subtype is involved in modulation of 5-HT release from vascular adrenergic nerve endings.

Materials and Methods

Perfusion of mesenteric vascular bed: The experiments were performed on the mesenteric vascular bed isolated from male Wistar rats, weighing 300 to 350 g. The animals were anesthetized with pentobarbital-Na (50 mg/kg, i.p.), and the mesenteric vascular bed was prepared for perfusion by the method of McGregor (8) as reported previously (5). The superior mesenteric artery was cannulated and gently flushed with Krebs-Ringer bicarbonate solution in order to eliminate blood in the vascular bed. The mesenteric vascular bed was separated from the intestine by cutting close to the intestinal wall. The isolated mesenteric vascular bed was placed on a 10-ml water-jacked organ bath maintained at 37°C and perfused with a modified Krebs-Ringer bicarbonate solution at a constant flow rate of 5 ml/min by means of a peristaltic pump (Atto, SJ-1215). The preparation was also superfused with the same solution at a rate of 0.5 ml/min to prevent drying. A modified Krebs-Ringer bicarbonate solution of the following composition (mmoles/liter) was used: NaCl, 120; KCl, 5.0; CaCl₂, 2.4; MgSO₄, 1.2; NaHCO₃, 25; EDTA, 0.027 and dextrose, 11 (pH 7.4). The solution was gassed with a mixture of 95% O₂-5% CO₂ before passage through a warming coil maintained at 37°C. Changes in the perfusion pressure were measured with a pressure transducer (Nihon Kohden, MPU-0.5A) and recorded on a polygraph (Nihon Kohden, RM-25).

Periarterial nerve stimulation (PNS) and infusion of NA: After allowing basal perfusion pressure to stabilize, the perfused mesenteric vascular bed was subjected to PNS and NA infusion. The PNS was performed for 30 sec at 5-min intervals using an automatic timer via bipolar platinum ring electrodes placed

around the superior mesenteric artery. Square wave pulses of 2 msec duration and supramaximal voltage (60 V) were delivered at 4, 8, 12 and 16 Hz by an electronic stimulator (Nihon Kohden, SEN 1101) with an isolator unit (Nihon Kohden, SS 101J). The neural basis of pressor response mediated by the stimulation of the periarterial adrenergic nerve was previously confirmed by abolition of the response after perfusion of guanethidine (1 μ M), tetrodotoxin (0.1 μ M) and phentolamine (0.1 μ M) and pretreatment with 6-hydroxydopamine (100 mg/kg, i.p. times 2) (5).

NA (0.5 nmol) was infused directly into the perfusate proximal to the arterial cannula with an infusion pump (Harvard, Model 975) for 10 sec in a volume of 0.05 ml.

Experimental protocols: After pressor responses to both the first PNS (4 to 16 Hz) and NA (0.5 nmol) infusion were obtained as the control, both the second PNS and NA infusion were carried out in the presence and absence of the agonist or antagonist. Thereafter, 5-HT was perfused in the absence of the drug for 15 min. After discontinuation of 5-HT perfusion, the preparation was perfused with Krebs-Ringer bicarbonate solution or Krebs-Ringer bicarbonate solution containing the drug for at least 30 min, and PNS, NA infusion and perfusion of 5-HT were performed in the absence or presence of the drug.

Measurement of tritium efflux: The isolated mesenteric vascular bed was perfused with Krebs-Ringer bicarbonate solution at a constant flow rate of 2.5 ml/min and superfused at 0.1 ml/min by means of the peristaltic pump. After a 30-min perfusion for stabilizing the perfusion pressure, the vascular bed was perfused and labelled with 0.1 μ M tritiated [1,2-³H(N)]-5-HT (24.2, 26.2 and 30.0 Ci/mmol) for 20 min. After rinsing for 90 min, the perfusate was collected in 2-min fractions. PNS was carried out for 1 min at 18-min intervals using an automatic timer. Rectangular pulses of 2 msec duration at 8 Hz and supramaximal voltage were given via platinum ring electrodes. Aliquots of 0.5 ml were added to 4.5 ml of scintillation fluid (Aquazol-2, New England Nuclear) and counted for 10 min with a scintillation counter (Aloka, LSC-

730). The perfusion of Krebs-Ringer bicarbonate solution containing the drugs was begun 10 min before and throughout the second PNS (S_2). In some experiments, yohimbine was perfused 20 min before the first PNS (S_1) through the rest of experiment, and Krebs-Ringer bicarbonate solution containing yohimbine and the drug were perfused 10 min before and throughout S_2 . After completion of the experiment, the mesenteric vasculature was weighed, digested with Soluene-100 (Packard Instrument) and counted for 10 min with the scintillation counter. The total tritium efflux was expressed as percentage of the amount of tritium in the tissue. The net efflux of tritium induced by PNS was determined by tritium efflux of two 2-min samples (during and after PNS) minus the spontaneous efflux just before PNS. The effect of drugs was expressed as the ratio between the net efflux produced by S_1 and the net efflux induced by S_2 .

Statistical analysis: Results were statistically analyzed using Student's *t*-test for group mean comparisons. A *P* value less than 0.05 was considered to be statistically significant.

Drugs: The following drugs were used: clonidine hydrochloride (gift from Nippon C.H. Boehringer Sohn), cocaine hydrochloride (Takeda), [$1,2\text{-}^3\text{H(N)}$]-5-HT creatinine sulfate (New England Nuclear), 5-HT hydrochloride (Sigma Chemical Co.), LY-53857 [4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl) 4, 6, 6A, 7, 8, 9, 10, 10A-octahydroindolo(4,3-FG)quinoline maleate] (gift from Eli Lilly), methoxamine hydrochloride (Mexan, Nihon Shinyaku), *l*-NA hydrochloride (Sigma Chemical Co.), prazosin hydrochloride (gift from Taito Pfizer) and yohimbine hydrochloride (Sigma Chemical Co.). Both 5-HT and NA were dissolved in 0.9% saline containing 0.1% ascorbic acid and stored in a freezer. On the day of the experiments, final dilutions of 5-HT and NA were made with Krebs-Ringer bicarbonate solution just before they were perfused or infused. All drugs were dissolved in distilled water and diluted in Krebs-Ringer bicarbonate solution gassed with a mixture of 95% O_2 -5% CO_2 before perfusion.

Results

Effect of treatment with 5-HT on pressor

Fig. 1. Typical record of effect of treatment with 5-hydroxytryptamine (5-HT) on pressor responses to periaarterial nerve stimulation (PNS) and infusion of noradrenaline (NA) in the perfused mesenteric vascular bed of the rat. A, pressor responses to the first PNS and NA infusion (Control); B, pressor responses to the second PNS and NA infusion (Before 5-HT); C, pressor responses to perfusion of 5-HT (10 μM) for 15 min; D, pressor responses to PNS, NA infusion and perfusion of 5-HT 30 min after 5-HT treatment (After 5-HT).

responses to PNS and NA infusion: As shown in Fig. 1A, the PNS (4 to 16 Hz) induced a frequency-dependent increase of perfusion pressure in the perfused mesenteric vascular bed. The responses to the second PNS at 12 and 16 Hz were slightly but significantly smaller than those to the first PNS (Figs. 1B and 2). The infusion of NA (0.5 nmol) also elevated the perfusion pressure. When the preparation was treated with 5-HT (10 μ M) for 15 min (Fig. 1C), the pressor response to PNS was greatly potentiated (Fig. 1D). However, the pressor response to exogenous NA was not significantly affected by 5-HT treatment. The potentiation was more pronounced at low frequencies of 4 and 8 Hz (Fig. 2).

Fig. 2. Effect of treatment with 5-hydroxytryptamine (5-HT) on pressor responses to periaarterial nerve stimulation (PNS) and infusion of noradrenaline (NA) in the perfused mesenteric vascular bed of rats. Control, pressor responses to the first PNS and NA infusion; Before 5-HT, pressor responses to the second PNS and NA infusion before perfusion of 5-HT (10 μ M); After 5-HT, pressor responses to PNS and NA infusion 30 min after 5-HT treatment. A dotted column in the right side represents the pressor response to 5-HT (10 μ M) in the absence of the drug. Vertical bars representing S.E.M. are shown except when contained within the symbol. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the pressor response to the second PNS (Before 5-HT).

Effect of 5-HT antagonist on pressor responses to PNS and NA infusion after 5-HT treatment: The selective 5-HT₂ receptor antagonist LY53857 (0.01 μ M) by itself was without effect on the pressor response to PNS and NA infusion. However, the pressor response to 5-HT (10 μ M) was greatly inhibited by the antagonist (Fig. 3). As shown in Fig. 3, the pressor response to PNS was not altered by 5-HT treatment when LY53857 (0.01 μ M) was present in the perfusion medium.

Effects of methoxamine and clonidine on pressor responses to PNS and NA infusion after 5-HT treatment: Methoxamine (1 μ M) by itself caused a small but significant

Fig. 3. Effect of LY53857 (0.01 μ M) on pressor responses to periaarterial nerve stimulation (PNS) and infusion of noradrenaline (NA) after treatment with 5-hydroxytryptamine (5-HT) (10 μ M) in the perfused mesenteric vascular bed of rats. Control, pressor responses to the first PNS and NA infusion in the absence of LY53857; LY53857, 0.01 μ M, pressor responses to the second PNS and NA infusion in the presence of LY53857 before perfusion of 5-HT; After 5-HT, pressor responses to PNS and NA infusion in the presence of LY53857 30 min after 5-HT treatment. A dotted column in the right side represents the pressor response to 5-HT (10 μ M) in the presence of LY53857. Vertical bars representing the S.E.M. are shown except when contained within the symbol. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the control. *** $P < 0.001$, compared with the pressor response to 5-HT alone.

potentiation of the pressor responses to both PNS (4 and 8 Hz) and NA infusion (Fig. 4). The pressor response to PNS after 5-HT treatment was further potentiated in the presence of methoxamine (Fig. 4). However, there are no significant differences between control and methoxamine (0.1 and 1 μ M) as shown in Fig. 7.

Clonidine (1 μ M) by itself reduced significantly the pressor response to PNS (8 to 16 Hz) and slightly that to NA infusion (Fig. 5). After 5-HT treatment, the pressor response to PNS but not that to NA infusion was greatly potentiated in the presence of clonidine (1 μ M) (Fig. 5). The potentiation

was more pronounced at low frequencies of 4 and 8 Hz (Fig. 5). However, the potentiation of pressor response to PNS after 5-HT treatment did not occur when clonidine (1 μ M) and LY53857 (0.01 μ M) were present in the perfusion medium (Fig. 6). As shown in Fig. 7, the degree of the potentiating effect of clonidine (1 μ M) was significantly greater than that of the control.

Both methoxamine (0.1 and 1 μ M) and clonidine (0.1 and 1 μ M) did not alter the pressor response to perfusion of 5-HT (10 μ M).

Effects of clonidine, methoxamine and cocaine on the PNS-evoked tritium efflux:

Fig. 4. Effect of methoxamine (1 μ M) on pressor responses to periarthral nerve stimulation (PNS) and infusion of noradrenaline (NA) after treatment with 5-hydroxytryptamine (5-HT) (10 μ M) in the perfused mesenteric vascular bed of rats. Control, pressor responses to the first PNS and NA infusion in the absence of methoxamine; methoxamine, 1 μ M, pressor responses to the second PNS and NA infusion in the presence of methoxamine before perfusion of 5-HT; After 5-HT, pressor responses to PNS and NA infusion in the presence of methoxamine 30 min after 5-HT treatment. A dotted column in the right side represents the pressor response to 5-HT (10 μ M) in the presence of methoxamine. Vertical bars representing S.E.M. are shown except when contained within the symbol. * P <0.05, ** P <0.01, compared with the control. * P <0.05, ** P <0.01, compared with the pressor response to the second PNS (Before 5-HT).

Fig. 5. Effect of clonidine (1 μ M) on pressor responses to periarthral nerve stimulation (PNS) and infusion of noradrenaline (NA) after treatment with 5-hydroxytryptamine (5-HT) (10 μ M) in the perfused mesenteric vascular bed of rats. Control, pressor responses to the first PNS and NA infusion in the absence of clonidine; clonidine, 1 μ M, pressor responses to the second PNS and NA infusion in the presence of clonidine before perfusion of 5-HT; After 5-HT, pressor responses to PNS and NA infusion in the presence of clonidine 30 min after 5-HT treatment. A dotted column in the right side represents the pressor response to 5-HT (10 μ M) in the presence of clonidine. Vertical bars representing S.E.M. are shown except when contained within the symbol. * P <0.05, ** P <0.01, *** P <0.001, compared with the control. ** P <0.01, *** P <0.001, compared with the pressor response to the second PNS (Before 5-HT).

Fig. 6. Effect of combined treatment with clonidine (1 μ M) and LY53857 (0.01 μ M) on pressor responses to periarterial nerve stimulation (PNS) and infusion of noradrenaline (NA) after treatment with 5-hydroxytryptamine (5-HT) (10 μ M) in the perfused mesenteric vascular bed of rats. Control, pressor responses to the first PNS and NA infusion in the absence of clonidine and LY53857; 1 μ M clonidine + 0.01 μ M LY, pressor responses to the second PNS and NA infusion in the presence of clonidine and LY53857 before perfusion of 5-HT; After 5-HT, pressor responses to PNS and NA infusion in the presence of clonidine and LY53857 30 min after 5-HT treatment. A dotted column in the right side represents the pressor response to 5-HT (10 μ M) in the presence of clonidine and LY53857. Vertical bars representing S.E.M. are shown except when contained within the symbol. * P < 0.05, ** P < 0.01, *** P < 0.001, compared with the control. *** P < 0.001, compared with the pressor response to 5-HT alone.

In the perfused mesenteric vascular bed labelled with [3 H]-5-HT, the PNS (8 Hz) evoked an increase of the tritium efflux in the perfusate. The PNS-evoked tritium efflux was abolished by tetrodotoxin (0.1 μ M), guanethidine (0.1 μ M) and Ca^{++} -free Krebs-Ringer solution, indicating that the tritium efflux was produced by arterial adrenergic nerve stimulation (data not shown).

Clonidine (0.01 to 1 μ M) produced a concentration-dependent increase of the PNS-evoked tritium efflux, while cocaine at a concentration of 10 μ M inhibited the tritium

efflux. A significant difference from the control was found in either 1 μ M clonidine or 10 μ M cocaine. Methoxamine (0.1 to 10 μ M) was without effect on the PNS-evoked tritium efflux (Table 1).

Effects of yohimbine, prazosin and LY-53857 on the PNS-evoked tritium efflux: Yohimbine (0.1 to 1 μ M) facilitated significantly the PNS-evoked tritium efflux in the perfused mesenteric vascular bed labelled with [3 H]-5-HT in a concentration-dependent manner (Table 2). Prazosin at a concentration of 0.1 μ M and LY53857 at concentrations of 0.01 and 0.1 μ M were without effect on the PNS-evoked tritium efflux, while a high concentration of prazosin (1 μ M) increased significantly the PNS-evoked tritium efflux (Table 2).

Effects of clonidine and cocaine on the PNS-evoked tritium efflux in the presence of yohimbine: As shown in Table 3, clonidine (1 μ M) failed to produce an increase of the PNS-evoked tritium efflux in the presence of yohimbine (1 μ M). Also, cocaine (10 μ M) did not induce a decrease in the PNS-evoked tritium efflux, and instead, it tended to increase the efflux in the presence of yohimbine.

Discussion

The present study demonstrated that when the mesenteric vascular bed was treated with 5-HT, the pressor response to PNS was greatly potentiated without significantly affecting the response to exogenously administered NA. Since the potentiation of the neurogenic pressor response seen after 5-HT treatment was blocked by LY53857, a selective 5-HT₂ receptor antagonist (9), 5-HT₂ receptors appear to be involved in this potentiation. Furthermore, in the preparation labelled with [3 H]-5-HT, PNS evoked an increase of the tritium efflux. The PNS-evoked tritium efflux was abolished by tetrodotoxin and by elimination of calcium from the perfusion medium but not by LY-53857. These results confirmed the previous findings that 5-HT was taken up and released from the vascular adrenergic nerve by adrenergic nerve stimulation (5). The present study and the previous report provide additional evidence that the released 5-HT by PNS has an ability to produce the vasocon-

Fig. 7. Effect of methoxamine, clonidine and LY53857 on pressor responses to periaarterial nerve stimulation (8 Hz) after treatment with or without 5-hydroxytryptamine (5-HT) (10 μ M) in the perfused mesenteric vascular bed of rats. Open columns (5-HT untreated) represent responses in the absence (control) or presence of the drug before perfusion of 5-HT and closed columns (5-HT treatment) indicate responses in the absence (control) or presence of the drug 30 min after treatment with 5-HT. Ordinate represents percentage of response in the absence or presence of each drug before 5-HT treatment. Each value is the mean of 5 to 8 experiments and vertical bars show S.E.M. Asterisks indicate significant difference from the control.

Table 1. Effects of α -adrenoceptor agonists and a monoamine uptake inhibitor on [3 H]-efflux induced by periaarterial nerve stimulation (PNS, 8Hz) of the perfused mesenteric vascular bed labelled with [3 H]-5-hydroxytryptamine in rats

	n	Conc. (μ M)	Ratio (S_2/S_1)	Significance
Control	20		0.91 $\pm$ 0.03	
Agonist				
Methoxamine	5	0.1	0.80 $\pm$ 0.07	N.S.
	5	1	0.96 $\pm$ 0.04	N.S.
	4	10	0.78 $\pm$ 0.15	N.S.
Clonidine	5	0.01	0.88 $\pm$ 0.15	N.S.
	5	0.1	1.08 $\pm$ 0.11	N.S.
	7	1	1.29 $\pm$ 0.12	P<0.001
Uptake inhibitor				
Cocaine	6	10	0.52 $\pm$ 0.12	P<0.001

Values are shown as means $\pm$ S.E. and the ratio of the second PNS (S_2)/the first PNS (S_1).

striction, which is mediated by postsynaptic 5-HT₂ receptors. It has been reported that 5-HT amplifies the response to NA which is exogenously added or released from adrenergic nerves and that the amplifying effect of 5-HT is due to interaction with 5-HT₂ receptors (10). Therefore, it seems likely that the potentiation of the pressor response to

PNS seen after 5-HT treatment results from a combined effect of the vasoconstriction of the released 5-HT by itself and the amplifying action of the released 5-HT on the response to neuronally released NA.

The enhanced pressor response to PNS after 5-HT treatment was not altered by methoxamine, a selective α_1 -adrenoceptor

Table 2. Effects of α -adrenoceptor antagonists and a 5-hydroxytryptamine (5-HT) receptor antagonist on [3 H]-efflux induced by periarterial nerve stimulation (PNS, 8Hz) of the perfused mesenteric vascular bed labelled with [3 H]-5-hydroxytryptamine in rats

	n	Conc. (μ M)	Ratio (S_2/S_1)	Significance
Control	20		0.91 $\pm$ 0.03	
Antagonist				
Prazosin	6	0.1	0.95 $\pm$ 0.14	N.S.
	8	1	1.34 $\pm$ 0.18	P<0.01
Yohimbine	5	0.1	1.37 $\pm$ 0.14	P<0.001
	6	1	1.64 $\pm$ 0.13	P<0.001
LY53857	5	0.01	0.93 $\pm$ 0.20	N.S.
	4	0.1	0.93 $\pm$ 0.15	N.S.

Values are shown as means $\pm$ S.E. and the ratio of the second PNS (S_2)/the first PNS(S_1).

Table 3. Effects of clonidine and cocaine on [3 H]-efflux induced by periarterial nerve stimulation (PNS, 8Hz) in the presence of yohimbine in the perfused mesenteric vascular bed of rats labelled with [3 H]-5-hydroxytryptamine

	n	Conc. (μ M)	Ratio (S_2/S_1)	Significance
Yohimbine alone	6	1	0.65 $\pm$ 0.03	
Yohimbine + Clonidine	6	1	0.61 $\pm$ 0.07	N.S.
Yohimbine + Cocaine	6	10	0.91 $\pm$ 0.15	N.S.

Values are shown as means $\pm$ S.E. and the ratio of the second PNS(S_2)/the first PNS(S_1). Perfusion of yohimbine was begun 20 min before S_1 through the rest of the experiment.

agonist. Also, methoxamine had no effect on the PNS-evoked tritium efflux in the preparation labelled with [3 H]-5-HT. These results suggest that presynaptic α_1 -adrenoceptors are not involved in modulation of the 5-HT release. This is supported by the present findings that prazosin, a selective α_1 -adrenoceptor antagonist, at a concentration of 0.1 μ M but not 1 μ M did not modify the PNS-evoked tritium efflux. The increased PNS-evoked tritium efflux by a high concentration of prazosin (1 μ M) may be due to the inhibition of the negative feedback mechanism mediated by presynaptic α_2 -adrenoceptors, since prazosin at concentrations more than 0.1 μ M has been shown to antagonize α_2 -adrenoceptors (11, 12).

The present experiment showed that the PNS-evoked tritium efflux in the preparation labelled with [3 H]-5-HT was facilitated by yohimbine, an α_2 -adrenoceptor antagonist, which is well-known to produce an increase in the neurotransmitter (NA) release to electrical stimulation of adrenergic nerves by interrupting the feedback control of neurotransmitter release mediated by presynaptic

α_2 -adrenoceptors (6, 7). Therefore, it seems likely that the PNS-evoked 5-HT release from vascular adrenergic nerves is also regulated by the negative feedback mechanism mediated by presynaptic α_2 -adrenoceptors.

The preferential α_2 -adrenoceptor agonist, clonidine has been shown to inhibit the neurotransmitter (NA) release to electrical stimulation of vascular adrenergic nerves, leading to a reduction of vasoconstrictor response to nerve stimulation (6, 7, 13). This effect is generally considered to be due to activation of presynaptic α_2 -adrenoceptors. In the present experiment, clonidine in the preparation untreated with 5-HT markedly inhibited the pressor response to PNS with a small reduction of the response to exogenous NA, indicating that the inhibition probably results from the decreased neurotransmitter (NA) release by activating presynaptic α_2 -adrenoceptors. On the contrary, clonidine enhanced further the potentiation of pressor response to PNS seen after 5-HT treatment. This effect of clonidine may be attributed to the increased 5-HT release from adrenergic nerve endings,

since clonidine had little effect on the vasoconstrictor response to 5-HT and to NA after 5-HT treatment, and this effect of clonidine did not occur in the presence of the 5-HT₂ receptor antagonist. This contention is further supported by the present findings that in the preparation labelled with [³H]-5-HT, clonidine produced a concentration-dependent increase of the PNS-evoked tritium efflux, which was antagonized by yohimbine. Thus, these results raise a question that activation of presynaptic α_2 -adrenoceptors does not decrease the PNS-evoked 5-HT release, unlike the neurotransmitter (NA) release.

The PNS-evoked tritium efflux in the preparation labelled with [³H]-5-HT was inhibited by cocaine. This effect was the surprising finding, since cocaine is well-known to increase the stimulation-evoked overflow of the neurotransmitter (NA) as well as [³H]-NA by preventing the reuptake mechanism into adrenergic nerve endings. How can we explain the effect of clonidine and cocaine on the PNS-evoked 5-HT release from vascular adrenergic nerves? Two possibilities arise. Firstly, the PNS-evoked 5-HT release from adrenergic nerves may depend upon an amount of endogenous NA which is simultaneously released by PNS. In the presence of cocaine, neurally released NA may accumulate in the neuro-effector junction to operate the feedback process mediated by presynaptic α_2 -adrenoceptors, leading to a reduction of the PNS-evoked 5-HT release. In fact, the present experiment showed that cocaine in the presence of yohimbine failed to inhibit the PNS-evoked tritium efflux and instead, facilitated the efflux. It should be noted that the potentiation of the pressor response to PNS after 5-HT treatment was more pronounced at low frequencies (4 and 8 Hz). This probably is because the amount of NA evoked by low frequencies is not enough to operate the feedback mechanism, which inhibits a release of 5-HT. Secondly, although it is difficult to explain the effect of clonidine on the 5-HT release by PNS, we wish to propose the following hypothesis. Recently, Bond et al. (14) suggested that cholinergic nerves of the guinea-pig ileum

have two adrenergic receptors: one recognizing both clonidine and NA, the other recognizing only NA. This may lead to speculation that adrenergic nerve endings in the mesenteric artery also have two populations of α_2 -adrenoceptors, i.e., α_{2A} and α_{2B} , which are able to be stimulated by neuronally released NA. The activation of α_{2A} receptors may inhibit the release of neurotransmitter (NA) and that of α_{2B} receptors may inhibit the release of a false transmitter such as 5-HT taken up, respectively. If clonidine acts only at α_{2A} receptors to reduce endogenous NA release, the local concentration of endogenous NA in the vicinity of α_{2B} would decrease. This may lead to reduction of the feedback inhibition of the 5-HT release and subsequently result in the facilitation of the 5-HT release. Further study is needed to clarify the mechanism of presynaptic α_2 -adrenoceptor-mediated modulation of the neurotransmitter and 5-HT release from vascular adrenergic nerves.

In conclusion, it is suggested that the 5-HT release from vascular adrenergic nerves is modulated by presynaptic α_2 -adrenoceptors. It is hypothesized that vascular adrenergic nerves may have two populations of presynaptic α_2 -adrenoceptors.

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