An amplifying effect of exogenous and neurally stored 5-hydroxytryptamine on the neurogenic contraction in rat tail artery

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- 1 The interactions between sympathetic neuroeffector transmission and 5-hydroxytryptamine (5-HT) were investigated in segments of rat isolated tail artery.
- 2 Contractile responses to field stimulation of the artery segments were abolished by tetrodotoxin (3×10^{-7} M). A subthreshold concentration of acutely applied exogenous 5-HT (10^{-8} M) markedly enhanced the contractions induced by sympathetic nerve stimulation, through an action on postjunctional 5-HT₂-receptors.
- 3 The amplifying effect of 5-HT involved an enhanced influx of extracellular calcium into the smooth muscle cells. In contrast, the neurogenic contractions in vessels not exposed to 5-HT were not dependent on extracellular calcium.
- 4 The adrenergic component of the amplified response involved postjunctional α_1 - but not α_2 - adrenoceptor activation.
- 5 Exposure of the vessels to 5-HT (5×10^{-7} M) for 30 min resulted in uptake of the amine into the perivascular sympathetic nerves, as demonstrated by immunohistochemistry. After chemical sympathectomy with 6-hydroxydopamine *in vitro* or *in vivo*, or surgical sympathectomy, there was little or no uptake.
- 6 Exposure to 5-HT followed by repeated washing resulted in an enhancement of the neurogenic contraction, which was still fully tetrodotoxin-sensitive. The enhanced response was blocked by ketanserin (10^{-8} M) and prevented by the presence of the 5-HT uptake blocker, paroxetine (3×10^{-8} M), during the period of exposure to 5-HT.

Introduction

An amplifying effect of 5-hydroxytryptamine (5-HT) on the contractions induced by sympathetic nerve stimulation or exogenous noradrenaline (NA) has been reported in many blood vessels (for review see Van Nueten *et al.*, 1988; de la Lande, 1989). This effect of 5-HT usually, but not always (Chang & Owman, 1989), involves activation of 5-HT₂-receptors on the vascular smooth muscle. It has been suggested that the potentiating effect of 5-HT on the NA-induced contractions plays an important role in various diseases including essential hypertension (Vanhoutte, 1982) and cerebral and coronary vasospasm (Owman, 1988; Cohen, 1988), where vascular smooth muscle adrenoceptors may become exposed to enhanced circulating or local concentrations of 5-HT, perhaps even released from the perivascular sympathetic nerves after prior uptake.

The underlying mechanism of the amplifying effect of 5-HT is not fully understood. Recent studies, investigating the interactions between NA and 5-HT (Meehan *et al.*, 1988), or phenylephrine and 5-HT (Marwood, 1988), where all agents were exogenously applied, indicate that the 5-HT-induced enhancement of extracellular calcium influx to the vascular smooth muscle cells may play an important role. In an attempt to elucidate further the role of calcium in the amplifying effect, the interaction between exogenously applied 5-HT and endogenous NA released from the sympathetic nerves by transmural field stimulation was studied in the rat tail artery.

In various vascular preparations, including cerebral (Levitt & Duckles, 1986; Saito & Lee, 1987; Owman, 1988), coronary (Cohen, 1985; 1988), mesenteric (Kawasaki & Takasaki, 1984) and ear arteries (Meehan *et al.*, 1986), neuronal uptake of 5-HT may play a role in the regulation of vascular tone. 5-HT that accumulates in perivascular sympathetic nerves can be

released by transmural field stimulation, and causes contraction of the smooth muscle, as demonstrated in canine coronary (Cohen, 1985; 1988), rabbit basilar (Saito & Lee, 1987) and rat mesenteric arteries (Kawasaki & Takasaki, 1984). In the present study we investigated whether such uptake also occurs in perivascular nerves of the rat tail artery and, if so, whether such accumulated 5-HT, upon release, could amplify the effect of neurally released NA in a manner similar to exogenously applied 5-HT.

Methods

Male Sprague-Dawley rats weighing 300–400 g were killed by diethyl ether anaesthesia. A segment (1 cm in length) of the intermediate part of the tail artery (located from 6 to 7 cm from the proximal end of the tail) was dissected out and immediately placed in an ice-cold Krebs-Ringer buffer solution. Within 30 min of dissection, segments (2 mm in length) of the vessels were mounted on two L-shaped metal holders between platinum electrodes (0.3 mm in diameter, 4 mm apart, placed parallel to the vessel) in an organ bath for measurement of isometric circular muscle contractions following exposure to various agents and electrical field stimulation (Hardebo *et al.*, 1986). The organ bath contained a Krebs-Ringer buffer solution of the following composition (in mM): NaCl 118, KCl 4.5, CaCl₂ 1.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.0 and glucose 6.0. The bath temperature was kept at 37°C and the pH at 7.35 by continuous slow aeration with 88.5% O₂ + 11.5% CO₂.

After mounting, the segments were subjected to a passive load of 4–5 mN (depending on vessel size) and then allowed to stabilize for 40–60 min. The passive load applied was of a magnitude that allowed a maximum contractile response of the vessels as tested in separate control experiments. Agents used to enhance or block the responses to electrical stimulation were added to the bath 15 min beforehand, except for 5-HT 10^{-8} M which was added 2 min beforehand. Incubations

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for 30 min in the presence of 5-HT 5×10^{-7} M (in some experiments together with paroxetine 3×10^{-8} M) and of prostaglandin $F_{2\alpha}$ 5×10^{-6} M were also performed. Several segments of each tail artery from the individual animals were used.

Electrical field stimulation

Electrical stimulation with 10 s trains of 9 V monophasic square wave impulses were delivered from a Grass S44 stimulator. The current during the pulses was 72 mA. The frequency was set at 6 Hz, and the duration of individual impulses was 0.1 ms. These stimulus parameters were optimal to produce a fully tetrodotoxin (TTX, 3×10^{-7} M)-sensitive and thus purely neurogenic response (see Szabó & Hardebo, 1990).

Denervations

In some experiments the vessels were acutely denervated *in vitro* with 6-hydroxydopamine (6-OHDA) according to the method of Aprigliano & Hermsmeyer (1976). The solution in the organ bath was replaced for 10 min by a bicarbonate-free solution containing 6-OHDA $30 \mu\text{g ml}^{-1}$ and reduced glutathione $20 \mu\text{M}$. The O_2/CO_2 mixture aerating the bath was turned off during this treatment. Following this denervation procedure, the segments were allowed to recover in normal aerated solution for 150 min. *In vivo* denervation was achieved by i.p. injection of 6-OHDA (50 mg kg^{-1} , dissolved in saline containing 0.1% ascorbic acid) two days before and 100 mg kg^{-1} one day before the rats were killed. Surgical denervation was performed by transection of both ventral collector nerves according to Sittiracha *et al.* (1987) 2 cm from the proximal end of the tail. The animals were killed 4 days later and a segment 5 cm long, starting 3 cm distal to the section level, was dissected out.

Immunohistochemistry

After incubation for 30 min in 37°C Krebs-Ringer solution with 5×10^{-7} M 5-HT, or without 5-HT (control), the tail arteries were fixed for 2 h in a fixative solution containing 2% formaldehyde and 15% of a saturated aqueous picric acid solution in 0.2 M phosphate buffer (pH 7.2). After fixation, they were thoroughly rinsed for 24 h in a Tyrode solution containing 10% sucrose at 4°C , followed by sectioning in a cryostat at $12 \mu\text{m}$ thickness and mounted on chromalum-coated slides. The indirect immunohistochemical procedure of Coons *et al.* (1955) was used. The specimens were incubated in a humid chamber of 4°C for 24 h with antiserum against 5-HT raised in rabbit (generous gift from H.W.M. Steinbusch, Department of Pharmacology, Free University, Amsterdam, The Netherlands) at a dilution of 1:250 or with antiserum against tyrosine hydroxylase raised in a rabbit (lot no. 1012, Eugene Tech, Allendale, U.S.A.) at a dilution of 1:320. After repeated rinsing in 0.1 M phosphate buffer solution containing 0.3% Triton X-100 for 30 min the vessels were incubated with porcine anti-rabbit IgG conjugated with fluorescein isothiocyanate (DAKOPATTS, Denmark) at a dilution of 1:20 for 45 min at room temperature. The slides were then rinsed twice in phosphate buffer solution containing 0.3% Triton X-100 before mounting in a mixture of the buffer and glycerin (1:1). They were examined in a Zeiss fluorescence microscope equipped with incident illumination.

Drugs

The following drugs were used: ascorbic acid (Sigma), Bay K 8644 (Bayer), D-600 (methoxyverapamil, gift from Knoll AG), ketanserin (Janssen), α,β -methylene-ATP (Sigma), neuropeptide Y (NPY; Peninsula), reduced glutathione (Sigma), paroxetine hydrochloride (Ferrosan), phenylephrine hydrochloride (Winthrop), prazosin hydrochloride (Pfizer), prostaglandin $F_{2\alpha}$ (Astra), rauwolscine hydrochloride (Roth), 5-HT

creatinine sulphate (Sigma), tetrodotoxin (TTX, Sigma). Prazosin was dissolved in a few drops of methanol, followed by 10% lactic acid, in a stock solution that was further diluted with 0.9% NaCl solution. All other agents were dissolved in 0.9% NaCl solution.

Statistics

For statistical analyses, Student's *t* test was applied for paired and unpaired data. The indicated values are means $\pm$ s.e.mean.

Results

Characterization of the neurogenic response

The electrical field-induced contractions were totally abolished by TTX (3×10^{-7} M). The effect on the neurogenic responses of the α -adrenoceptor antagonists prazosin and rauwolscine, the 5-HT₂ receptor antagonist ketanserin, and D-600 (a blocker of potential-sensitive calcium channels) in untreated vessels are summarized in Figure 1a. Prazosin (5×10^{-8} M) and rauwolscine (10^{-6} M) significantly attenuated neurogenic responses in untreated vessels by 63 and 39%, respectively. A marked (88%) inhibition of neurogenic responses was produced by the combination of prazosin and rauwolscine and further addition of a high concentration of NPY (10^{-7} M) to pre-occupy junctional NPY receptors almost eliminated the response. Ketanserin (10^{-8} M) and D-600 (10^{-6} and 10^{-5} M) had no effect on the neurogenic responses in untreated vessels.

Neurogenic response in the acute presence of 5-hydroxytryptamine

The presence of 5-HT (10^{-8} M), added to the vessel chamber 2 min beforehand, enhanced the contractions induced by sympathetic nerve stimulation, while this concentration of 5-HT did not, on its own, alter the vascular tone (Table 1, Figure 2a). The neurogenic response was enhanced by 92% in the presence of 5-HT.

Ketanserin (10^{-8} M) or D-600 (10^{-5} M) eliminated the enhancement by 5-HT of the neurogenic contraction. A similar inhibition was observed after application of 5×10^{-8} M prazosin or 10^{-6} M rauwolscine (Figure 1b). The combined application of prazosin and rauwolscine did not totally inhibit the response, in contrast to the effect in untreated vessels. The combined presence of prazosin and rauwolscine did not cause a greater inhibition than prazosin alone, again contrasting to the effect in untreated vessels. However, a further inhibition of the response was obtained when D-600 was applied in combination with prazosin and rauwolscine (Figure 1b). This inhibition by the calcium antagonist was concentration-dependent, in contrast to the absence of effect in untreated vessels. Furthermore, the combined presence of the α -antagonists and D-600 caused a more complete inhibition of the response than D-600 alone. Thus, in the presence of 5-HT, all individual inhibitors or combinations of them caused significant reductions of the enhanced neurogenic response, whereas in untreated vessels only α -adrenoceptor antagonists caused significant reductions of the response.

Neurogenic response in the acute presence of other constrictors

To elucidate whether this enhancement was specific to 5-HT, the possible amplifying effect of various other constrictor agents on the neurogenic response was studied. The results are given in Table 1. A minor elevation of the extracellular potassium (from 5.5 mM to 10 mM) caused a significant enhancement of the contraction, without changing the resting tone.

Figure 1 Effects of α_1 - and α_2 -adrenoceptor antagonists, a 5-HT₂-receptor antagonist and a calcium channel-blocker, and the presence of a high concentration of neuropeptide Y (NPY), on the neurogenic contractions induced by transmural nerve stimulation in the rat tail artery. (a) Effects of the blockers and NPY on the response in untreated vessels. (b) Effects of the blockers on the response in the presence of 5-hydroxytryptamine (5-HT) 10^{-8} M. Significant differences from the control value, i.e. in untreated (control) vessels, and also between different treatments in (a), * $P < 0.05$ and ** $P < 0.001$, respectively. Significant differences between the contractile response in the presence of 5-HT (10^{-8} M) alone and in the combined presence of 5-HT and the blocking agents, and also between different treatments in (b): † $P < 0.05$, †† $P < 0.01$ and ††† $P < 0.001$ respectively. NS = nonsignificant. All reported values represent % of the response in untreated (control) vessels, which is set as 100%.

Table 1 Effects of various agents on the contractions (in mN) induced by transmural nerve stimulation on the resting tone in rat tail artery

Agent	n	Contraction before the application of agent (mN)	Contraction after the application of agent (mN)	Direct effect of the contractile agent on tone (mN)
5-HT 10^{-8}	17	3.50 ± 0.36	$5.93 \pm 0.49^{***}$	none
K ⁺ 10 mM	20	3.35 ± 0.38	$4.83 \pm 0.72^{***}$	none
α,β -methylene-ATP 10^{-6} M	20	3.66 ± 0.44	$5.24 \pm 0.66^{***}$	$1.43 \pm 0.32^{**}$
Phenylephrine 10^{-8} M	6	3.22 ± 0.53	3.08 ± 0.44	none
Phenylephrine 10^{-7} M	6	3.22 ± 0.53	3.31 ± 0.53	none
Phenylephrine 10^{-6} M	12	3.08 ± 0.40	3.50 ± 0.30	$2.65 \pm 0.38^{***}$
Prostaglandin F _{2a} 5×10^{-7} M	17	3.50 ± 0.41	3.73 ± 0.51	0.09 ± 0.05
Prostaglandin F _{2a} 5×10^{-6} M	14	3.73 ± 0.53	$6.66 \pm 0.89^{***}$	$2.04 \pm 0.25^{***}$
Bay K 8644 10^{-8} M	8	3.22 ± 0.73	$5.78 \pm 1.32^{**}$	none

Significant differences ** $P < 0.01$ and *** $P < 0.001$, respectively from control values or from the resting tone. n = number of vessels.

Figure 2 Representative individual experiments on neurogenic contractions induced by transmural nerve stimulation (9 V, 72 mA, 0.1 ms pulses at 6 Hz for 10 s) in the rat tail artery. The effect on the responses of: (a) acute exposure to 5-hydroxytryptamine (5-HT) 10^{-8} M; (b) elevation of the extracellular potassium concentration from 5.5 to 10 mM; (c) exposure to α,β -methylene-ATP 10^{-6} M; (d) Bay K 8644 10^{-8} M.

The depolarizing purinoceptor agonist α,β -methylene-ATP (10^{-6} M) significantly elevated the resting tone and significantly enhanced the neurogenic contractions. Phenylephrine did not affect the neurogenic response, either at low concentrations (10^{-8} – 10^{-7} M), which did not affect the resting tone, or at a higher concentration (10^{-6} M) which caused contraction. Prostaglandin $F_{2\alpha}$, at a concentration (5×10^{-7} M) that caused a slight contraction had no effect on the neurogenic response, whereas a concentration of 5×10^{-6} M, that caused a more marked contraction, strongly enhanced the neurogenic response. Activation of potential-sensitive calcium channels by Bay K 8644, at a concentration of 10^{-8} M, markedly enhanced the neurogenic contraction (77%), without affecting the resting

Figure 3 Individual experiment on a rat tail artery showing: (a) the absence of an effect of ketanserin, 10^{-8} M, on the neurogenic response to transmural nerve stimulation; (b) the stable contraction during exposure to 5-HT 5×10^{-7} M for 30 min, its reversal after repeated washing (W), and the enhanced neurogenic response after the incubation; (c) the partial blockade by ketanserin 10^{-8} M of the enhanced neurogenic response. Note the change in time scale in the intermediate part of the tracing in (b).

tone. Typical tracings of some of these responses are shown in Figure 2.

Neurogenic response after preincubation with 5-hydroxytryptamine

In the second part of the study the vessels were incubated for 30 min in 5×10^{-7} M 5-HT, followed by repeated washouts and a return of tone to the baseline. Following this treatment the neurogenic response was enhanced (Table 2, Figure 3b). Ketanserin (10^{-8} M) markedly attenuated the enhancement and now the response was not significantly different from that before incubation with 5-HT (Table 2). When the 5-HT uptake blocker paroxetine (3×10^{-8} M) was present during the incubation with 5-HT, no significant enhancement of the subsequent neurogenic response was observed. Further, the difference in enhancement between 5-HT incubation with and without uptake blocker was significant.

In the presence of prostaglandin $F_{2\alpha}$ (5×10^{-6} M) a significant enhancement of the neurogenic response was observed (Table 1), which was abolished after washout (Table 2).

Neurogenic response in the acute presence of 5-HT or after preincubation with 5-HT following sympathetic denervation

Immunohistochemical staining for 5-HT showed no or very sparse specific fluorescence in untreated vessels (Figure 4b),

Table 2 Effect of incubation of the vessels with 5-hydroxytryptamine (5-HT) 5×10^{-7} M for 30 min, incubation with 5-HT in the presence of paroxetine 3×10^{-8} M, the effect of application of ketanserin 10^{-8} M or tetrodotoxin (TTX) 3×10^{-7} M after incubation with 5-HT, or incubation with prostaglandin $F_{2\alpha}$ 5×10^{-6} M for 30 min on the electrically induced neurogenic contractions in rat tail artery

Incubation for 30 min	n	Contraction before incubation (mN)	Contraction after incubation (mN)	Contraction after incubation as % of contraction before incubation
5-HT 5×10^{-7} M	15	3.00 ± 0.32	$5.86 \pm 0.72^{***}$	$212 \pm 23\%^{***}$
5-HT 5×10^{-7} M + paroxetine 3×10^{-8} M	7	3.77 ± 0.47	4.37 ± 0.70	$123 \pm 20\%^{\dagger}$
5-HT 5×10^{-7} M followed by ketanserin 10^{-8} M	10	3.17 ± 0.29	3.46 ± 0.33	$115 \pm 12\%^{\dagger\dagger}$
5-HT 5×10^{-7} M followed by TTX 3×10^{-7} M	8	3.79 ± 0.63	0 ^{***}	0 ^{***} †††
Prostaglandin $F_{2\alpha}$ 5×10^{-6} M	6	3.27 ± 1.04	3.42 ± 1.09	$102 \pm 2\%^{\dagger\dagger}$

Significant differences between contractions before and after incubation with 5-HT: $^{***}P < 0.001$. Significant differences (in %) between the enhanced response after incubation with 5-HT alone and the other treatments. $^{\dagger}P < 0.05$, $^{\dagger\dagger}0.01$ and $^{\dagger\dagger\dagger}0.001$, respectively. n = number of vessels.

Figure 4 (a) 5-Hydroxytryptamine (5-HT)-immunofluorescent nerve fibres in the adventitial-medial border in a cross-section of a rat tail artery following incubation for 30 min with 5-HT 5×10^{-7} M. (b) Control vessel with no or very few visible 5-HT-containing fibres. (c) Vessel from *in vitro* 6-hydroxydopamine-treated rat following incubation in the presence of 5-HT, few 5-HT-containing fibres are visible. Bar = 100 μ m.

whereas after 30 min incubation in 5-HT (5×10^{-7} M), dense 5-HT-immunofluorescence was observed in the adventitial-medial border (Figure 4a). After *in vivo* treatment with 6-OHDA or section of both ventral collector nerves, the majority of sympathetic nerves, as demonstrated by tyrosine hydroxylase immunoreactivity, had disappeared, and only few 5-HT-immunofluorescent fibres were observed after prior incubation (Figure 4c).

The effects of the various denervation procedures on the neurogenic response are summarized in Table 3. In vessels not exposed to 5-HT, only a weak, TTX-sensitive response

remained after 6-OHDA treatment *in vivo*, and there was no response at all after *in vitro* treatment. After treatment, no facilitation of the weak response was seen in the presence of 5-HT (Table 3a) or after incubation with 5-HT for 30 min (Table 3b). However, after section of both ventral collector nerves, a procedure which reduces the sympathetic nerve density by about 80% (Sittiracha *et al.*, 1987), a marked potentiation of the remaining weak response was seen both in the presence of 5-HT and after incubation. The seemingly larger relative enhancement of the response after 5-HT treatment compared to that in untreated vessels is, in all probability, due to a smaller response prior to 5-HT exposure in denervated than in intact vessels.

Discussion

Presence of 5-hydroxytryptamine extracellularly

The results of the present study support previous findings in rat tail artery that 5-HT in subthreshold concentrations enhances contractions induced by sympathetic nerve stimulation, and that this effect of 5-HT involves activation of post-junctional 5-HT₂ receptors (Medgett *et al.*, 1984; Xiao & Rand, 1989). In this vessel, 5-HT causes depolarization of the smooth muscle cell membrane (Neild & Kotecha, 1987) and influx of extracellular calcium through potential-sensitive calcium channels (Chang & Owman, 1987). The present finding of a marked inhibition of the 5-HT-induced potentiation by the potential-sensitive calcium channel blocker D-600 indicates that such a mechanism is also operating for the 5-HT-induced enhancement of neurogenic responses in this artery. Only intracellular calcium mobilization appears to be involved in the neurogenic response in untreated vessels, since the calcium channel blocker did not inhibit the response, in accordance with findings by Medgett & Rajanyagam (1984). It has been shown that activation of postjunctional α_1 -adrenoceptors, but not α_2 -adrenoceptors in this vascular smooth muscle causes mobilization of intracellular calcium (see Timmermans & Thoolen, 1987). Accordingly, the α_1 -adrenoceptor antagonist prazosin inhibited the response to a greater extent than the α_2 -adrenoceptor antagonist rauwolscine in untreated vessels.

The facilitating effect on neurogenic responses by vasoactive agents other than 5-HT may also be due to depolarization and calcium influx through potential-sensitive channels into the smooth muscle cells. It has been shown in rat tail artery and rabbit ear artery that a modest elevation of the potassium concentration (Neild & Kotecha, 1987) or exposure to Bay K 8644 (Cheung & Mackay, 1986) or α,β -methylene-ATP (Neild & Kotecha, 1986) causes a small depolarization of the smooth muscle membrane. Recent investigations have shown that even a small depolarization of the cell membrane substantially alters the availability of extracellular calcium to the smooth muscle cells by altering the opening-closing state of the calcium channels in the membrane. In this way, contractions to exogenously applied NA are facilitated (Nelson *et al.*, 1988). Prostaglandin F_{2a} and the α_1 -adrenoceptor agonist phenylephrine, in concentrations that did not contract the vessels, did not facilitate the neurogenic response, probably because the receptors on which they act are not linked to enhanced calcium influx (Uski & Andersson, 1984; van Zwieten & Timmermans, 1987).

Recent studies on the potentiating effect of 5-HT on the contractions caused by exogenously applied adrenoceptor agonists have suggested that the effect is dependent upon extracellular calcium, since it is counteracted by diltiazem or lowered extracellular calcium (Meehan *et al.*, 1988; Xiao & Rand, 1989). These results are in agreement with our findings, that D-600 abolished the enhanced part of the neurogenic response, similarly to the effect of ketanserin. Since the present combined application of the calcium channel blocker and α -

Table 3 Effect in rat tail artery of *in vitro* 6-hydroxydopamine (6-OHDA) denervation, *in vivo* 6-OHDA denervation and section of both ventral collector nerves, on the enhanced neurogenic contractile response normally seen (a) in the presence of 5-hydroxytryptamine (5-HT) 10^{-8} M and (b) after incubation with 5-HT 5×10^{-7} M for 30 min

a			
Denervation	n	Contraction (mN) untreated vessels	Contraction (mN) in the presence of 5-HT (10^{-8} M)
<i>In vitro</i> 6-OHDA denervation	4	0	0
<i>In vivo</i> 6-OHDA denervation	8	0.31 ± 0.35	0.34 ± 0.23
Surgical denervation	5	1.10 ± 0.39	$3.04 \pm 1.08^*$
b			
Denervation		Contraction (mN) before incubation	Contraction (mN) after incubation for 30 min with 5-HT (5×10^{-7} M)
<i>In vitro</i> 6-OHDA denervation	4	0	0
<i>In vivo</i> 6-OHDA denervation	8	0.25 ± 0.18	0.28 ± 0.26
Surgical denervation	10	0.84 ± 0.29	$2.38 \pm 0.48^{**}$

Significant differences from control values. * $P < 0.05$ and ** 0.01, respectively. n = number of vessels.

adrenoceptor antagonists caused a more pronounced inhibition of the contraction than either blocker alone, our results support the proposal that the binding sites on the calcium channels for blockers like verapamil and D-600 represent the overlapping area (or common binding site) for 5-HT₂-receptors and α_1 -adrenoceptors involved in the amplifying effect (Marwood, 1988). Thus our results support an interaction between 5-HT and NA on the postjunctional receptors, which involves an enhanced calcium influx through potential-sensitive channels when NA is of endogenous origin. However, since there was a significant (30%) remaining contraction in the presence of 5-HT, even if both α -blockers and D-600 were applied, additional amplifying mechanisms may be present.

While the neurogenic response in untreated vessels was markedly blocked after combined α_1 - and α_2 -adrenoceptor blockade, a less pronounced blockade by these two antagonists was obtained in the presence of 5-HT 10^{-8} M. To explain this, an additional amplifying mechanism involving postjunctional α_2 -adrenoceptors and 5-HT₂-receptors might be present. However, this is made unlikely by the finding that combined blockade by α_1 - and α_2 -adrenoceptor antagonists in vessels exposed to 5-HT was not more effective than α_1 -blockade alone, in contrast to the situation in untreated vessels. A possible interaction between postjunctional 5-HT₂-receptors and γ -receptors, which have been proposed to exist in several vascular beds including the rat tail artery (for review see Hirst & Edwards, 1989), may be considered. If they exist, they play only a very minor role in sympathetic neuroeffector mechanisms in the rat tail artery, since the small component of the neurogenic contractile response remaining in untreated vessels after combined α_1 - and α_2 -adrenoceptor blockade was almost fully attributable to activation of NPY-receptors. Therefore, the additional amplifying mechanism by 5-HT might possibly consist of an interaction between exogenous 5-HT and NPY, released upon nerve stimulation. Previous studies on the rat tail artery has excluded the possibility of endogenous cholinergic, purinergic and prostanoid mechanisms in the tonic response to field stimulation at the present stimulation parameters (Szabó & Hardebo, 1990).

Presence of 5-hydroxytryptamine in perivascular sympathetic nerves

The second part of the study demonstrates that incubation of the vessels with 5-HT for 30 min followed by washout enhances the neurogenic contractions. The enhanced response was still fully TTX-sensitive. The effect is due to release of 5-HT from the perivascular sympathetic nerves after prior

uptake, since (1) 5-HT could be clearly demonstrated by immunohistochemistry in perivascular nerves after prior exposure to 5-HT (but not or to a very minor extent in untreated vessels); (2) the perivascular sympathetic nerves, together with the 5-HT-containing fibres in vessels that had been exposed to 5-HT, were markedly reduced in number after *in vivo* 6-OHDA treatment; (3) no enhanced contractile response was seen following 6-OHDA treatment; (4) the presence of the 5-HT uptake blocker, paroxetine, counteracted this enhancement; and (5) ketanserin significantly reduced the response. Upon incubation, 5-HT may also accumulate extraneuronally in arteries (Fukuda *et al.*, 1986). Release from such stores is not responsible for the presently observed facilitation of the neurogenic response, since the enhanced contraction was fully TTX-blockable.

The surgical denervation procedure that was used eliminates only about 80% of the perivascular sympathetic fibres (Sittiracha *et al.*, 1987). There was uptake of 5-HT into the remaining sympathetic nerves, and it enhanced the small contractile response to electrical field stimulation. Furthermore, the denervation causes a supersensitivity of postjunctional receptors to 5-HT (see Szabó & Hardebo, 1990). The lack of enhancement of the neurogenic contractions after incubation with prostaglandin F_{2 α} clearly shows that the enhancement is not due to an altered calcium influx (or rearrangement of intracellular calcium) in the smooth muscle cells secondary to a prolonged contraction.

In conclusion, the study demonstrates a capacity of perivascular sympathetic nerves in the rat tail artery to accumulate 5-HT. Upon release, this neuronal 5-HT is able to enhance the contractions caused by stimulation of the adrenergic nerves to an extent similar to exogenously applied 5-HT in a concentration of 10^{-8} M. The enhanced response results from an opening by 5-HT of potential-sensitive calcium channels. Upon exposure of the vessel wall to 5-HT such a mechanism may contribute to an enhanced contractile response when the perivascular sympathetic nerves become activated. This may occur when platelets disrupt in conjunction with thrombosis formation, especially at sites with endothelial damage (Cohen, 1988). In hypertension, where the vascular postsynaptic 5-HT receptors are supersensitive (Van Nueten & Janssens, 1986), such a neurogenic contractile response may become even larger.

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