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European Journal of Pharmacology 338 (1997) 97–104

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Serotonin inhibits nitric oxide synthesis in rat vascular smooth muscle cells stimulated with interleukin-1

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Received 23 June 1997; revised 18 August 1997; accepted 29 August 1997

Abstract

We investigated the effects of serotonin (5-hydroxytryptamine; 5-HT) on nitric oxide (NO) synthesis in vascular smooth muscle cells. We measured the production of nitrite, a stable metabolite of NO, and the expression of inducible NO synthase protein in cultured rat vascular smooth muscle cells. Incubation of the cultures with interleukin-1β (10 ng/ml) caused a significant increase in nitrite production. 5-HT inhibited nitrite production by interleukin-1β-stimulated vascular smooth muscle cells in a concentration-dependent manner (10^{-8}–10^{-5} M). 5-HT-induced inhibition of nitrite production was accompanied by decreased inducible NO synthase protein accumulation in vascular smooth muscle cells. Addition of the 5-HT_2 receptor antagonist ketanserin, but not the 5-HT_{1A} receptor antagonist spiroxatrine, inhibited the effect of 5-HT. On the other hand, the 5-HT_2 receptor agonist α-methyl-5-HT, but not the 5-HT_{1A} receptor agonist (±)-8-hydroxy-2-(di-*n*-propylamino) tetralin, decreased interleukin-1β-induced nitrite production by vascular smooth muscle cells. 5-HT significantly increased protein kinase C activity in vascular smooth muscle cells, and the protein kinase C inhibitor calphostin C dose-dependently abolished the effect of 5-HT on nitrite production. After protein kinase C activity was functionally depleted by treatment of cells with phorbol 12-myristate 13-acetate for 24 h, the effect of 5-HT was abolished. These results indicate that 5-HT acts on 5-HT_2 receptors and inhibits NO synthesis in interleukin-1β-stimulated vascular smooth muscle cells at least partially through a protein kinase C-dependent pathway. © 1997 Elsevier Science B.V.

Keywords: Nitrite; Interleukin-1; Atherosclerosis; Protein kinase C

1. Introduction

Nitric oxide (NO), an extensively characterized endothelium-derived relaxing factor, is a short-lived free radical which plays an important role as an intercellular messenger, participating in vascular homeostasis, neurotransmission, and antimicrobial defense. NO is synthesized from the amino acid L-arginine in a reaction catalyzed by two enzymes, Ca^{2+}- and calmodulin-dependent constitutive NO synthase (cNOS) and Ca^{2+}- and calmodulin-independent inducible NO synthase (iNOS, or type II NOS). Two isoforms of constitutive NO synthase have been identified in endothelial cells (ecNOS, or type III NOS) and brain (nNOS, or type I NOS). Inducible NO synthase has been identified in endotoxin- and cytokine-treated neutrophils, hepatocytes, endothelial cells and myocardium (Moncada et al., 1991; Nathan and Xie, 1994; Ikeda et al., 1996). Its activity is also induced in aortic rings and cultured vascular smooth muscle cells by cytokines and endotoxin (Dinerman et al., 1993). NO inhibits platelet aggregation (Radomski et al., 1987a), proliferation of vascular smooth muscle cells (Grag and Hassid, 1989), and leukocyte adhesion to endothelial cells (Niu et al., 1994). Therefore, inducible NO synthase induction in vascular smooth muscle cells may play a role in local vascular injury associated with atherosclerosis.

Serotonin (5-hydroxytryptamine; 5-HT), a decarboxylation derivative of the amino acid tryptophan, is a naturally occurring vasoactive substance, and is a major secretory product of activated platelets. The wide variety of behavioral and physiological functions mediated by 5-HT is reflected in the numerous receptor subtypes (Hoyer et al.,

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1994). These receptors have been classified depending on their signal transduction mechanism: 5-HT_1 and 5-HT_5 subtypes as adenylyl cyclase inhibitors, 5-HT_2 as a phospholipase C stimulator, and 5-HT_4, 5-HT_6, and 5-HT_7 as adenylyl cyclase activators, are all members of the G protein-coupled receptors. The 5-HT_3 subtype is a 5-HT gated channel. In the cardiovascular system, multiple effects of 5-HT are mainly reflected by 5-HT_1 and 5-HT_2 receptors (Frishman et al., 1995). 5-HT is known to enhance vascular smooth muscle cell proliferation (Nemecek et al., 1986; Vankova and Grunmald, 1987) and contractility (Roth et al., 1984; Nakaki et al., 1985), and platelet aggregation (Vanhoutte and Lüscher, 1986). Recently, it has been shown that 5-HT also stimulates the expression of thrombin receptors in vascular smooth muscle cells (Schini-Kerth et al., 1996). Therefore, 5-HT may also play important roles in the progression of local vascular injury associated with atherosclerosis. However, there have been no reports concerning the effects of 5-HT on the synthesis of NO, another modulator of vascular contraction and proliferation, in vascular smooth muscle cells. In the present study, we investigated the effects of 5-HT on NO synthesis in cultured rat vascular smooth muscle cells.

2. Materials and methods

2.1. Materials

5-HT was purchased from Wako (Osaka, Japan). Human recombinant interleukin-1β (specific activity approx. 2×10^7 u/mg) was a gift from Otsuka Pharmaceuticals (Tokushima, Japan). The 5-HT_{1A} receptor antagonist spiroxatrine, 5-HT_2 receptor antagonist ketanserin, 5HT_{1A} receptor agonist ($\pm$)-8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) and 5-HT_2 receptor agonist α-methyl-5-HT were purchased from Research Biochemicals International (Natick, MA, USA). A monoclonal anti-mouse inducible NO synthase antibody, which cross-reacts against rat inducible NO synthase, was obtained from Transduction (Lexington, KY, USA). Calphostin C was purchased from Kyowa Hakko Kogyo (Tokyo, Japan). Phorbol 12-myristate 13-acetate was purchased from Sigma (St. Louis, MO, USA). All other chemicals used of the highest grade commercially available.

2.2. Cell culture

Primary cultures of vascular smooth muscle cells were obtained from the media of thoracic aortae of Sprague-Dawley rats (200–250 g), as described previously (Ikeda et al., 1991). The cells were grown in Dulbecco's minimum essential medium (DMEM) (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The cultures were harvested twice a week by treatment with 0.125% trypsin and passaged at a 1:3 ratio in 100 mm culture dishes. A typical growth experiment was performed using cultured cells at passage levels of 5–10. Cells (3×10^4 /ml) were plated in 24-well or 100 mm culture dishes in DMEM, supplemented as described above, and allowed to grow to sub-confluence for 24–48 h, after which they were pre-incubated in DMEM containing 0.5% fetal bovine serum and supplemented with insulin (5 μg/ml) and transferrin (5 μg/ml) for 24 h, and used for the experiments described below.

This investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institute of Health (NIH Publication No. 85–23, revised 1985).

2.3. Measurement of nitrite

NO production by the cultured cells was determined by measuring the nitrite content of the culture medium. Vascular smooth muscle cells plated in 24-well dishes were incubated in DMEM containing 0.5% fetal bovine serum at 37°C. The nitrite contents of culture media were determined by mixing 0.5 ml of medium with an equal volume of Griess reagent (1 part 0.1% naphthylethyl–enediamine dihydrochloride to 1 part 1% sulfanilamide in 5% phosphoric acid) (Green et al., 1982). The absorbance at 550 nm was measured and the nitrite concentration was determined by interpolation of a calibration curve of standard sodium nitrite concentrations against absorbance. After washing, cells were dissolved in 0.2 ml of 1% sodium dodecyl sulfate and used for protein assay (BCA protein assay kit; Pierce, Rockford, IL) with bovine serum albumin as a standard. Nitrite levels were corrected by protein contents and data are shown as nmol per mg protein. Average protein contents per well were 0.212 ± 0.014 mg ($n = 6$).

2.4. Assay for inducible NO synthase protein

The expression of inducible NO synthase protein was analyzed by immunoblotting with an anti-inducible NO synthase antibody (Koide et al., 1993). Briefly, cells were lysed in a buffer containing 50 mM Tris–HCl, pH 7.5, 1 mM EDTA, 1 μM leupeptin, 1 μM pepstatin A, 0.1 mM phenylmethylsulfonyl fluoride and 1 M dithiothreitol, and sonicated. The homogenates were then centrifuged at $100\,000 \times g$ for 20 min, and the supernatants (60 μg protein) were subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The separated proteins were electrophoretically transferred onto nitrocellulose membranes, and the resultant blots were incubated with the anti-mouse inducible NO synthase antibody for 2 h followed by peroxidase-labeled anti-mouse immunoglobulin G (IgG) for 1 h. Peroxidase-labeled proteins were detected using the enhanced chemiluminescence detection system (Amersham, Amersham, UK) on X-ray film.

2.5. Measurement of protein kinase C activity

Cells grown in 24-well dishes were incubated with serum-free DMEM for 24 h. After washing twice with phosphate-buffered saline, cells were exposed to 5-HT for approx. 20 min at 37°C. The reaction was stopped by addition of 0.1 ml of extraction solution (20 mM Tris–HCl, 0.5 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 25 μg/ml aprotinin and leupeptin, pH 7.5). Cell extracts were centrifuged at $1500 \times g$ for 5 min. The supernatant was then incubated with 25 μM of synthetic peptide (4–14 amino acids of bovine myelin basic protein; MBP-(4–14)) (Sigma) (Yasuda et al., 1990), and reaction mixture containing 20 mM Tris–HCl (pH 7.5), 5 mM magnesium acetate, 0.1 mM $CaCl_2$, 0.5 μg phosphatidyl serine, 50 ng diolein, and 50 μM [γ-^{32}P]ATP (specific activity; 10 Ci/mmol) for 10 min at 30°C. The reaction products were placed on P-81 paper (Whatman, Maidstone) and washed three times with 20 ml of ice-cold 10% phosphoric acid. The radioactivity was counted with a liquid scintillation counter (Aloka LSC-671, Tokyo). Specific radioactivity was obtained by subtracting the radioactivity of the synthetic peptide-free reaction from the synthetic peptide-directed radioactivity. Protein kinase C activity was represented as pmol of ATP incorporated per mg protein of cell extracts for 1 min.

2.6. Statistical analysis

Data are expressed as means ± S.E.M. of four samples, which represented at least three separate experiments. Differences were examined by one-way analysis of variance combined with Scheffe's test, and values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Effects of 5-HT on nitrite production

We first investigated the time course of the effect of 5-HT on nitrite production by vascular smooth muscle cells. As shown in Fig. 1, interleukin-1β (10 ng/ml) stimulated nitrite production by vascular smooth muscle cells in a time-dependent manner. Nitrite accumulation stimulated by interleukin-1β was significantly inhibited by 5-HT (10^{-6} M). After a 24 h incubation, the level of interleukin-1β-stimulated nitrite accumulation in the presence of 5-HT was approximately 70% of that in the absence of 5-HT.

Fig. 2 shows the concentration–response effect of 5-HT. 5-HT inhibited nitrite production by interleukin-1β-stimulated vascular smooth muscle cells in a concentration-dependent manner (10^{-8}–10^{-5} M). 5-HT alone did not affect the basal level of nitrite production.

3.2. Effects of 5-HT on inducible NO synthase protein expression

We next examined whether 5-HT inhibited interleukin-1β-induced nitrite production at the inducible NO synthase protein level. As shown in Fig. 3, unstimulated vascular smooth muscle cells showed no detectable inducible NO synthase protein expression. Incubation with interleukin-1β (10 ng/ml) for 24 h induced the expression of inducible NO synthase protein, and its effect was suppressed in the presence of 5-HT (10^{-6} M).

3.3. Effects of 5-HT receptor antagonists and agonists

As shown in Fig. 4, the 5-HT_2 receptor antagonist ketanserin concentration-dependently (10^{-8}–10^{-6} M) abolished the inhibitory effect of 5-HT on interleukin-1β-induced nitrite production by vascular smooth muscle cells. On the other hand, the 5-HT_{1A} receptor antagonist spiroxatrine did not affect the effect of 5-HT.

We also investigated the effect of 5-HT receptor agonists on nitrite production by interleukin-1β-stimulated vascular smooth muscle cells. The 5-HT_2 receptor agonist α-methyl-5-HT inhibited nitrite production in a concentration-dependent manner (10^{-8}–10^{-5} M) (Fig. 5), while the 5-HT_{1A} receptor agonist 8-OH-DPAT showed no inhibitory effect on nitrite production by interleukin-1β-stimulated vascular smooth muscle cells (data not shown).

3.4. Involvement of protein kinase C

The above observations suggested that 5-HT_2 receptors might mediate the inhibitory effect of 5-HT on nitrite

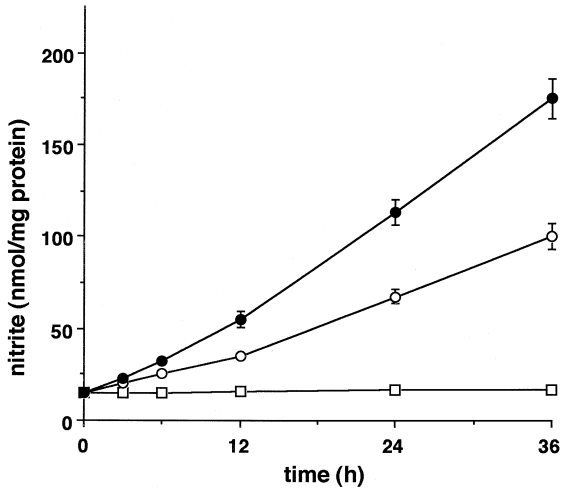

Fig. 1. Time-course of nitrite production by interleukin-1β-stimulated vascular smooth muscle cells in the presence or absence of 5-HT. Vascular smooth muscle cells were unstimulated (open squares), or stimulated with interleukin-1β (10 ng/ml; closed circles) or interleukin-1β plus 5-HT (10^{-6} M; open circles) for various periods as indicated. Nitrite accumulation in culture medium was measured as described in Section 2. Values were normalized to protein content per dish. Means ± S.E.M. are shown ($n = 4$).

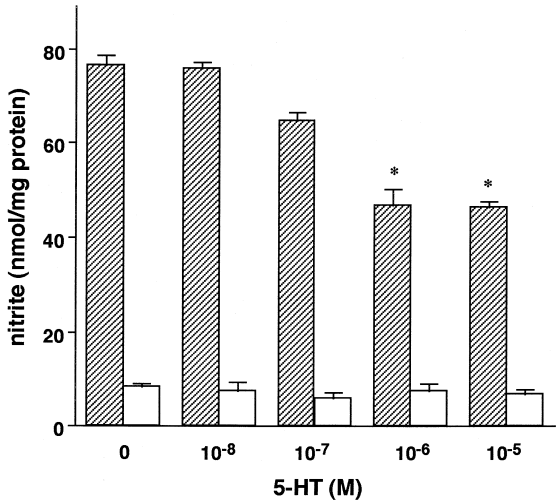

Fig. 2. Concentration-dependency of the inhibitory effect of 5-HT on nitrite production. Vascular smooth muscle cells were incubated for 24 h with (hatched bars) or without (open bars) interleukin-1β (10 ng/ml) in the presence of various concentrations of 5-HT (10^{-8}–10^{-5} M) as indicated. Nitrite accumulation in culture medium was measured. Values were normalized to protein content per dish. Means ± S.E.M. are shown ($n = 4$). $^{*}P < 0.05$ compared with controls not exposed to 5-HT.

production. 5-HT_2 receptors are coupled to activation of protein kinase C (De Courecells et al., 1985; Conn et al., 1986), and it has been shown that protein kinase C activation decreases NO synthesis in cytokine-stimulated vascular smooth muscle cells (Geng et al., 1994). Therefore, to investigate the involvement of protein kinase C in the effect of 5-HT on nitrite production, we first measured protein kinase C activity in vascular smooth muscle cells stimulated with 5-HT. As shown in Fig. 6, the activity was significantly increased 3–5 min after addition of 5-HT (10^{-6} M), and returned to the basal level after 10 min.

We then tested the effects of the protein kinase C inhibitor calphostin C on the effect of 5-HT. As shown in Fig. 7, calphostin C dose-dependently (10^{-8}–10^{-6} M)

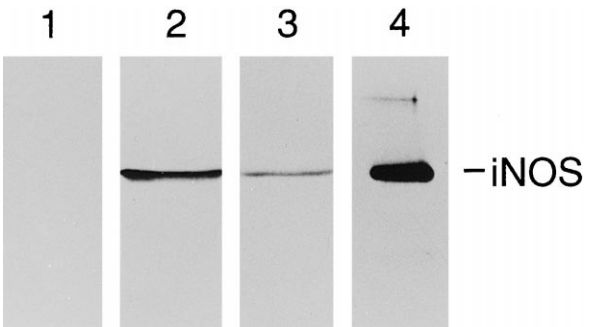

Fig. 3. Effects of 5-HT on inducible NO synthase protein accumulation. Cells were incubated for 24 h with interleukin-1β (10 ng/ml) with or without 5-HT (10^{-6} M). Cell extracts were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis followed by immunoblot analysis with the anti-inducible NO synthase (iNOS) antibody. Lane 1, control; lane 2, interleukin-1β; lane 3, interleukin-1β plus 5-HT; lane 4, positive control (mouse macrophage inducible NO synthase).

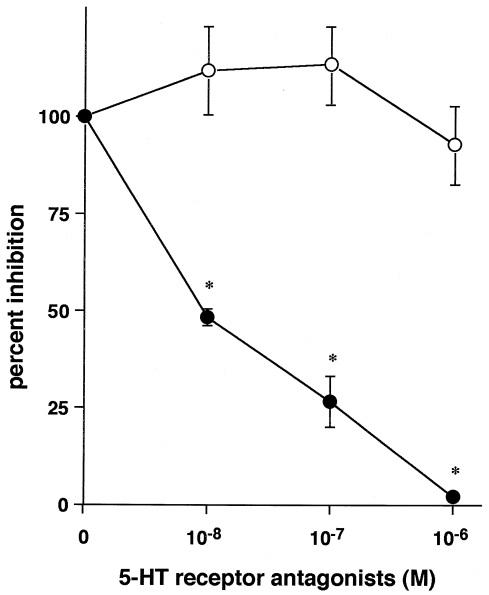

Fig. 4. Effects of 5-HT receptor antagonists on nitrite production by vascular smooth muscle cells treated with 5-HT. Various concentrations (10^{-8}–10^{-6} M) of 5-HT_{1A} receptor antagonist spiroxatrine (open circles) or 5-HT_2 receptor antagonist ketanserin (closed circles) were added to interleukin-1β (10 ng/ml) plus 5-HT (10^{-6} M)-stimulated vascular smooth muscle cells for 24 h. Values represent percent inhibition of 5-HT-mediated effects. 100% inhibition of nitrite accumulation by 5-HT represents 63.8 ± 4.8 nmol/mg protein. Data are means ± S.E.M. of four samples. $^{*}P < 0.05$ compared with control samples not exposed to the antagonists.

abolished the effect of 5-HT on interleukin-1β-induced nitrite production by vascular smooth muscle cells.

We further examined the effect of 5-HT in control and protein kinase C-depleted cells. It has been shown that

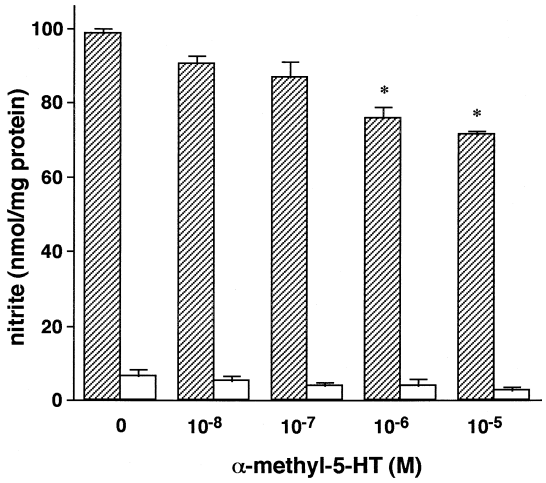

Fig. 5. Concentration-dependent effects of the 5-HT_2 receptor agonist on nitrite production by vascular smooth muscle cells. Vascular smooth muscle cells were incubated for 24 h with (hatched bars) or without (open bars) interleukin-1β (10 ng/ml) in the presence of various concentrations (10^{-8}–10^{-5} M) of the 5-HT_2 receptor agonist α-methyl-5-HT. Nitrite accumulation in culture medium was measured. Values were normalized to protein content per dish. Data are means ± S.E.M. of four samples. $^{*}P < 0.05$ compared with control samples.

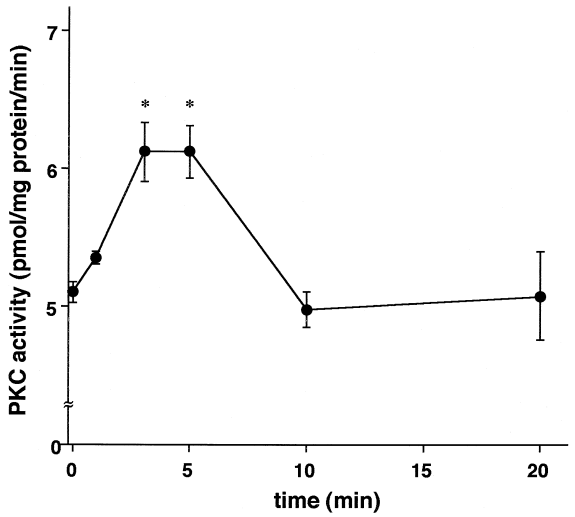

Fig. 6. Time course of changes in protein kinase C activity in vascular smooth muscle cells stimulated with 5-HT. Protein kinase C (PKC) activity was measured using a synthetic bovine myelin basic protein peptide (MBP-(4-14)) as described in Section 2. Values are represented as pmol of ATP incorporated per mg protein of cell extracts for 1 min. Data are means ± S.E.M. of four samples. $^{*}P < 0.05$ compared with 0 min samples.

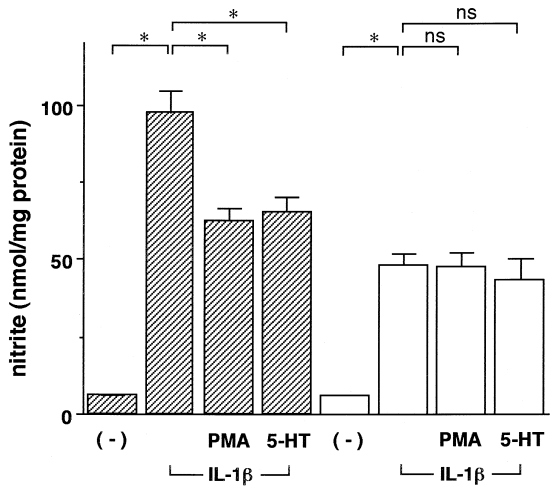

Fig. 8. Effects of 5-HT on nitrite accumulation in protein kinase C-depleted vascular smooth muscle cells. Cells pretreated (open bars) or not treated (hatched bars) with phorbol 12-myristate 13-acetate (PMA; 10^{-6} M) for 24 h were exposed to interleukin-1β (IL-1β; 10 ng/ml), interleukin-1β plus phorbol 12-myristate 13-acetate (10^{-7} M), interleukin-1β plus 5-HT (10^{-6} M), or vehicle (–) for a further 24 h. Data are means ± S.E.M. of four samples. $^{*}P < 0.05$.

protein kinase C activity in vascular smooth muscle cells is downregulated by pretreatment with phorbol 12-myristate 13-acetate (Griendling et al., 1989). Thus, cells were exposed to phorbol 12-myristate 13-acetate (10^{-6} M) in 10% fetal bovine serum for 24 h and then incubated in 0.5% fetal bovine serum with 5-HT (10^{-6} M), interleukin-1β (10 ng/ml), and/or phorbol 12-myristate 13-acetate (10^{-7} M) for a further 24 h. As shown in Fig. 8, in control cells not preincubated with phorbol 12-myristate 13-acetate, nitrite levels were significantly increased 24 h after addition of interleukin-1β. Addition of 5-HT or fresh phorbol 12-myristate 13-acetate reduced nitrite accumulation in interleukin-1β-stimulated cells. On the other hand, in cells preincubated with phorbol 12-myristate 13-acetate for 24 h, interleukin-1β still increased nitrite levels, but addition of fresh phorbol 12-myristate 13-acetate caused no change in nitrite levels, which is consistent with functional depletion of protein kinase C activity. The interleukin-1β-induced nitrite levels were not significantly affected by 5-HT in the protein kinase C-depleted cells.

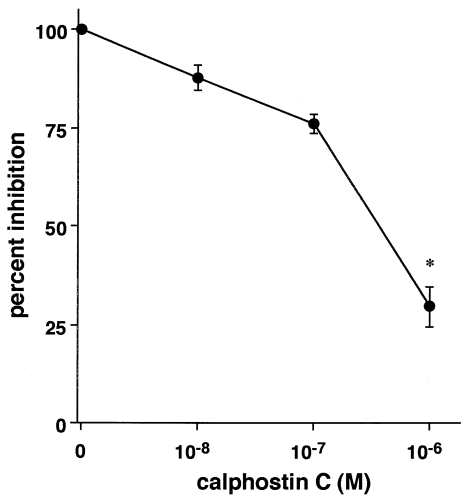

Fig. 7. Dose-dependent effects of calphostin C on the action of 5-HT. Calphostin C (10^{-8}–10^{-6} M) was added to the cultures in the presence of interleukin-1β (10 ng/ml) and 5-HT (10^{-6} M) for 24 h. Values represent the percent inhibition of 5-HT-mediated effects on nitrite production by calphostin C. 100% inhibition of nitrite accumulation by 5-HT represents 59.7 ± 3.2 nmol/mg protein. Data are means ± S.E.M. of four samples. $^{*}P < 0.05$ compared with control samples not exposed to calphostin C.

4. Discussion

The present study was designed to examine whether 5-HT affected NO synthesis in vascular smooth muscle cells. Although 5-HT by itself had no effect on nitrite accumulation, it significantly inhibited interleukin-1β-induced nitrite production in a time- and concentration-dependent manner. Furthermore, 5-HT inhibited interleukin-1β-induced inducible NO synthase protein accumulation.

5-HT receptors have been classified into various subtypes (Vanhoutte and Lüscher, 1986). In the cardiovascular system, multiple effects of 5-HT are mainly reflected by the activities of 5-HT_1 and 5-HT_2 receptors in many species (Cohen et al., 1981; Houston and Vanhoutte, 1981; Lee et al., 1989). In human arteries, 5-HT contracts coronary and mesenteric arteries by activating a mixture of both 5-HT_1-like and 5-HT_2 receptors, but it contracts cerebral arteries solely via 5-HT_1-like receptors (Parsons, 1991; Kaumann et al., 1993). Conversely, the mitogenic

and chemotactic effects of 5-HT on vascular smooth muscle cells are mediated predominantly by 5-HT_2 receptors. The present study showed that the inhibitory effect of 5-HT on NO synthesis was reduced in the presence of the 5-HT_2 receptor antagonist ketanserin, and that the 5-HT_2 receptor agonist α-methyl-5-HT inhibited interleukin-1β-induced nitrite production. In contrast, the 5-HT_{1A} receptor antagonist spiroxatrine did not affect the inhibitory effect of 5-HT, and the 5-HT_{1A} receptor agonist 8-OH-DPAT showed no effect on interleukin-1β-induced nitrite production. These findings suggest that the inhibitory effect of 5-HT on NO synthesis is mediated by 5-HT_2 receptors.

5-HT_2 receptor function is coupled to activation of phopholipase C (De Courecells et al., 1985; Conn et al., 1986) resulting in the liberation of the second messenger diacylglycerol which activates protein kinase C, and inositol triphosphate which mobilizes Ca^{2+} from intracellular stores (Cory et al., 1986; Pritchett et al., 1988; Julius et al., 1990; Van Obberghen-Schilling et al., 1991; Corson et al., 1992). In the present study, we observed that protein kinase C activity in vascular smooth muscle cells was significantly increased after addition of 5-HT. Furthermore, the protein kinase C inhibitor calphostin C dose-dependently abolished the inhibitory effect of 5-HT on nitrite production, and the inhibitory effect of 5-HT was also reduced in the protein kinase C-depleted cells. These results indicate that activation of protein kinase C mediates, at least partially, the effect of 5-HT on NO synthesis.

In healthy humans and animals, the plasma and vascular tissue concentration of 5-HT is maintained at low levels as a consequence of uptake and storage in platelets as well as the degradation by monoamine oxidases in endothelial cells and vascular smooth muscle cells. However, injury to the blood vessel wall resulting in the disruption or dysfunction of the endothelium (e.g., balloon angioplasty, atherosclerosis) disturbs these homeostatic mechanisms. Indeed, increased levels of 5-HT have been found locally in canine coronary arteries at sites of endothelial injury and stenosis (Ashton et al., 1986), in the canine coronary sinus after experimentally induced endothelial injury (Benedict et al., 1986), and also within the coronary bed of patients with unstable angina (Van den Berg et al., 1989). 5-HT is most likely released by mural thrombosis at sites of injury, since a close correlation has been found between the level of 5-HT and platelet deposition (Ashton et al., 1986; Benedict et al., 1986). Furthermore, micromolar concentrations of 5-HT can be reached after activation of platelet suspensions of cell density equivalent to those found in vivo (McGoon and Vanhoutte, 1984; Cohen, 1986). Thus, at sites of injury, the blood vessel wall may be chronically exposed to concentrations of platelet-derived 5-HT within the range necessary to evoke biological responses.

5-HT has important biological effects not only as a neurotransmitter but also as a vasoconstrictive substance. 5-HT also stimulates the mitogenesis of vascular smooth muscle cells (Lee et al., 1991; Corson et al., 1992; Hoyer et al., 1994; Lee et al., 1994) and fibroblasts (Mann, 1967; Boucek and Alvarez, 1970) and promotes the production of collagen by fibroblasts (Alto and Kulonen, 1972; Boucek et al., 1972). Thus 5-HT may contribute to the initiation and progression of myointimal arterial lesions characteristic of atherosclerosis. In addition, 5-HT facilitates platelet aggregation and fibrin formation to produce an acute thrombotic occlusion of the damaged artery. Recently, it has also been shown that 5-HT stimulates the expression of thrombin receptors in vascular smooth muscle cells, probably via 5-HT_2 activation of protein kinase C and tyrosine kinases (Schini-Kerth et al., 1996). These findings suggest that 5-HT could also accelerate the evolution of occlusive coronary thrombi.

On the other hand, several lines of evidence from both in vitro and in vivo studies have suggested a role of NO as an anti-atherogenic factor. NO inhibits aggregation of platelets (Radomski et al., 1987a) and restores blood flow in the balloon-injured artery (Yan et al., 1996). Diminished NO production accelerates coronary artery disease by promoting interactions between platelets and the vessel wall through loss of NO-mediated platelet inhibition (Radomski et al., 1987b). NO also inhibits proliferation of vascular smooth muscle cells (Grag and Hassid, 1989), production of cytokines by endothelial cells (Marcinkiewicz and Chain, 1993), and leukocyte adhesion to endothelial cells (Niu et al., 1994; Takahashi et al., 1996). Cayatte et al. (1994) reported that chronic inhibition of NO synthesis with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester accelerated neointimal formation in the aortae of hypercholesterolemic rabbits. Wang et al. (1994) reported that chronic administration of the NO precursor L-arginine prevented coronary atherogenesis in that rabbit model. However, the potential role of excess NO derived from inducible NO synthase under pathological conditions including atherosclerosis is not fully characterized and somewhat controversial.

The present study revealed that 5-HT acts on 5-HT_2 receptors and inhibits NO synthesis in interleukin-1β-stimulated vascular smooth muscle cells at least partially through a protein kinase C-dependent pathway. These findings suggest that, in addition to its direct effects on vascular smooth muscle cell and platelet functions, 5-HT may promote the initiation and progression of atherosclerosis by inhibiting cytokine-induced NO production by vascular smooth muscle cells. However, further investigations are necessary to elucidate the mechanisms and conditions under which NO and 5-HT attenuate atherogenesis.

Acknowledgements

This study was supported by the Ministry of Education, Science, Sports and Culture, Japan (#8670821) and the Mitsukoshi Grant-in-Aid 1996.

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