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Peripheral 5-HT_{2A}-receptor-mediated formation of an inhibitor of atrial natriuretic peptide binding involves inflammation

Yumiko Tozawa [a,b,*], Kouji Matsushima [a]

[a] *Department of Molecular Preventive Medicine, School of Medicine, University of Tokyo, Tokyo, Japan*
[b] *Department of Social Gerontology, School of Health Sciences and Nursing, University of Tokyo, Tokyo, Japan*

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Abstract

A peripheral 5-HT_{2A} receptor-mediated hemodynamic change prompted formation of indole-2,3-dione, an endogenous inhibitor of atrial natriuretic peptide (ANP) receptor binding and G protein-mediated intracellular signaling (IC_{50}: 0.4 μM). This effect was significantly suppressed by dexamethasone, indomethacin and the 5-HT_2 receptor antagonists, ketanserin or ritanserin. 5-HT_{2A} receptor-mediated acute hemodynamic change was not modified significantly by indomethacin, prazosin or propranolol pretreatment. A tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, but not a dopamine β hydroxylase inhibitor, diethyldithiocarbamate, abolished the 5-HT_{2A} receptor-mediated increase in indole-2,3-dione. Exogenous indole-2,3-dione induced a significant increase in plasma catecholamine levels and decrease in urine volume. A 5-HT_{2A} receptor-mediated decrease in capillary flow may have caused an inflammatory process and peripheral sympathetic activation via ANP signaling inhibition. 3,4-dihydroxyphenylalanine (DOPA)/dopamine may contribute to the progression of inflammation or the generation of a precursor of indole-2,3-dione. The observation that indole-2,3-dione abolished angiotensin AT_1 receptor-mediated NADPH activation in both human umbilical vein endothelial cells and smooth muscle cells at 20 μM may suggest that sulfhydryl-reactive indole-2,3-dione could influence mitochondrial function and cellular redox states via flavoenzyme inhibition and/or regulation of dehydrogenase–oxidase conversion.

Keywords: Inflammation; Catecholamine; 5-HT (5-hydroxytryptamine, serotonin); Reactive oxygen species; Peripheral resistance; Indole-2,3-dione

1. Introduction

Elevated peripheral resistance is primarily involved in the pathogenesis of vasculopathy-based disease states such as hypertension, atherosclerosis, diabetes mellitus and disorders of aging, all of which share inflammation in and around vessel walls (Gerrity et al., 1979; Rajavashisth et al., 1990; Alexander, 1995). The association of reactive oxygen species-mediated formation of highly oxidized lipids and 3,4-dihydroxyphenylalanine (DOPA)/dopamine-quinones (Graham, 1978; Kuhn and Arthur, 1998; Basma et al., 1995) with inflammatory process has been well documented. Further oxidized products downstream of cysteinyl-dopamine quinone are known to be formed intracellularly and participate in the initial pathological process of inflammatory diseases (Li et al., 1998). Elevated peripheral resistance may prompt formation of highly oxidized compounds through inflammation.

Both sympathetic overactivity and increased production of reactive oxygen species in the microcirculation have been viewed as major contributors to elevated peripheral resistance: Because the sympathetic nervous system is the key factor in regulating vasoconstrictor tone, increased vascular resistance in the kidney, liver and splanchnic area induced by a defense reaction has been accounted for by regional autonomic activation (Green and Kepchar, 1959; Forsyth, 1971). However, it is known that norepinephrine-infused rats do not exhibit vascular reactive oxygen species generation. Increased vascular resistance in the kidney or liver may be ascribed, at least in part, to the higher ratio of capillaries to feed and larger arteries in these organs. On the other hand, the abolition by xanthine oxidase inhibition of elevated peripheral resistance concomitant with elevated arterial pressure in spontaneous hypertensive rats (Suzuki et al., 1998; Nakazono et al., 1991) makes the increased reactive oxygen species in capillaries a key factor in the

* Corresponding author. Department of Molecular Preventive Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel.: +81-3-5841-3434; fax: +81-3-5841-3393.

E-mail addresses: yumikot-tky@umin.ac.jp, yumiko.tozawa@pobox.com (Y. Tozawa).

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regulation of vascular resistance, as the superoxide-generating form of xanthine oxidase is located predominantly in capillaries. It may also be hypothesized that norepinephrine precursors from phenylalanine (Phe) or tyrosine (Tyr), but not norepinephrine itself, are the agents mediating the progression of inflammation in the microcirculation under conditions that decrease capillary flow.

How can substantial evidence be obtained that elevated peripheral resistance due to decreased capillary flow prompts a local inflammatory process?

5-HT_{2A} receptor stimulation increases peripheral resistance and arterial pressure (Martin, 1994; Rattigan et al., 1999). These effects are ascribed, at least in part, to 5-HT_{2A} receptor-mediated constriction of resistance arterioles or flow-limiting vasoconstriction of various vessels, including the renal bed (Martin, 1994). Of great importance is that the vasoconstrictive action of 5-HT_{2A} receptor agonists is similar to that of norepinephrine at doses reflecting levels found at sympathetic vasoconstrictor synapses and that it resembles high frequency sympathetic nerve stimulation, in that it induces a decreased capillary flow and a simultaneously increased vascular resistance concomitant with elevated arterial pressure through site-specific vasoconstriction (Rattigan et al., 1999). Evidence is accumulating that 5-HT_{2A} receptor-mediated flow-limiting vasoconstriction may result in local tissue ischemia (Martin, 1994). The pathological importance of 5-HT_{2A} receptors has been suggested by results of many studies: atherosclerosis greatly increases activity in response to 5-HT_{2A} receptor activation in various vascular beds so that a relatively benign vascular response to 5-HT is converted to flow-limiting constriction (Martin, 1994).

Indole-2,3-dione is a stress-related endogenous compound whose biosynthetic pathway remains unclear (Glover et al., 1988; Watkins et al., 1990; Medvedev et al., 1996; Tozawa et al., 1998). Because the most potent physiological action of endogenous indole-2,3-dione is the inhibition of atrial natriuretic peptide (ANP) receptor binding and G protein-mediated intracellular signaling (IC_{50}: 0.4aμM) according to previous studies (Medvedev et al., 1996), indole-2,3-dione may be implicated as a physiological regulator of regional sympathetic activity and transport system of the renal tubules. Considering that indole-2,3-dione is a highly electron-deficient compound and is increased several fold in hypertension (in urine and tissues), Parkinson's disease (in urine) (Hamaue, 2000) and cancer (in urine) (Maki, 1959), it may be hypothesized that it is an oxidized metabolite produced at inflammation sites under specific conditions. This study was aimed to investigate whether a peripheral 5-HT_{2A}-receptor-mediated decreased capillary flow induces indole-2,3-dione formation through inflammation and whether exogenous indole-2,3-dione modulates plasma catecholamine levels and urine volume. Of 5-HT_{2A} receptor agonists, α-methyl-serotonin acts specifically on peripheral 5-HT_{2A} receptors in arterioles and causes decreased capillary flow and increases total peripheral resistance without reduction of femoral blood flow (Rattigan et al., 1999). In addition, the effect of indole-2,3-dione on angiotensin AT_1 receptor-mediated NADPH oxidase activation was examined because electrophilic indole-2,3-dione may modify thiol groups essential to the activities of oxidoreductases, including NAD(P)H oxidizing flavoenzymes. The anticipated sulfhydryl reactivity of indole-2,3-dione may make it a regulator of mitochondrial function and cellular redox states via enzyme inhibition and/or conversion of dehydrogenase to oxidase (McKelvey et al., 1988).

2. Materials and methods

2.1. Animals

All procedures were approved by The Animal Research Committee and meet the Guideline for the Care and Use of Laboratory Animals of the School of Medicine, University of Tokyo. Male Wistar rats (Japan Biological Materials Center, Tokyo, Japan) weighing 300 g were housed in metabolic cages, had easy access to food pellets and tap water, and were maintained under a 12-h light cycle. Rat urine samples were collected everyday at 4:00 PM during the period of experiments. Blood pressure of conscious animals was monitored from the tail artery with a programmable sphygmomanometer (PS-200A: Riken Kaihatsu, Tokyo). For the measurement of femoral blood flow, the rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). An incision was made in the skin overlying the femoral vessels of the left leg. The femoral artery was separated from the femoral vein and saphenous nerve, and the epigastric vessels were ligated. A flow probe was positioned around the femoral artery just distal to the rectus abdominis muscle and connected to a flowmeter (Nihon Kohden, Tokyo). Femoral blood flow values were recorded by pen-writing oscillography (RJG-4024, Nihon Kohden) after smoothing with a resistance and capacitance filter with a time constant of 1 s. Femoral vascular resistance was calculated as mean arterial blood pressure in millimeters of mercury divided by femoral blood flow in milliliters per minute. The flow probe was regularly calibrated before implantation.

2.2. Reagents

α-Methyl-5-HT,1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), *m*-chlorophenylpiperazine (*m*-CPP), ketanserin tartrate, ritanserin (the Funakoshi Pharmaceutical), prazosin, yohimbine, reserpine, propranolol, diethyldithiocarbamate, indole-2,3-dione (Sigma) dexamethasone, indomethacin, lipopolysaccharide, α-methyl-*p*-tyrosine (Wako) were dissolved just prior to use in saline or 1% Tween 80 in saline. For use in high performance liquid chromatography(HPLC), HPLC-grade aceto-nitrile, methanol and ethyl acetate (Wako) were used. All other chemicals for indole-2,3-dione assay were of analytical grade. Dulbecco's modified Eagle medium (DMEM), Dulbecco's phosphate-buffered

saline (D-PBS) (GIBCO Laboratories) and 6-carboxy-2′,7′-dichloro-dihydrofluorescin diacetate (CH_2DCF-DA) (Molecular Probe) were used.

2.3. Extraction of indole-2,3,-dione

Rat urine (1 ml) was diluted with 5 ml of distilled water and acidified with 6 M HCl to pH 1. The urine sample was then heated for 10 min in a boiling water bath to solubilize the urine sediment. After cooling at room temperature, indole-2,3-dione was extracted with 10 ml of ethyl acetate. The organic layer was then evaporated under a stream of nitrogen and the residue was dissolved in 0.3 ml of methanol and then diluted with 5 ml of 50 mM potassium phosphate buffer, pH 7.4. Indole-2,3-dione was extracted using a disposable solid-phase column, the Mega Bond Elut C18 column (Varian, Harbor City, CA, USA) (Tozawa et al., 1999; Manabe et al., 1997) and the eluate was analyzed by reversed-phase HPLC as described previously.

2.4. HPLC analyses

Reversed-phase HPLC analyses were performed using a Hitachi 655A chromatograph (Hitachi, Tokyo, Japan), as described previously (Tozawa et al., 1999; Manabe et al., 1997). Partial purification of indole-2,3-dione was carried out using a Shodex ES-502C column (100 7.6 mm I.D., 9.0 μm particle size; Showa Denko, Tokyo, Japan) under the following conditions: mobile phase, 50 mM potassium phosphate buffer (pH 7.4)-acetonitrile (85:15, V/V); flow rate, 1.0 ml/min; 50 °C. The final HPLC analysis was carried out using a Kaseisorb LC ODS Super column (250 4.6 mm I.D., 5 μm particle size and 120 Å pore size; Tokyo Chemical Industries, Tokyo, Japan). The column was equilibrated with 50 mM potassium phosphate buffer (pH 7.4)-acetonitrile (85:15, V/V) at a flow rate of 1 ml/min. The fraction corresponding to indole-2,3-dione in the first-step analysis was injected directly onto the column. Separation was carried out at 50 °C and the eluate was monitored by UV detection at 240 nm.

2.5. Cell culture

ECs were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Cells were passaged twice per week by harvesting with trypsin: EDTA and seeding into 75-cm^2 flasks. For experiments, cells between passage levels 8 and 20 were seeded into 35-mm and 100-mm dishes, fed every other day, and used at confluence.

2.6. Intracellular reactive oxygen species measurement by confocal laser microscopy

ECs were plated at low density, grown for 72 h in culture media containing 10% fetal bovine serum. Cells were then washed with Dulbecco's phosphate-buffered saline (D-PBS), pH 7.4, and stimulated with each drug in the presence or absence of indole-2,3-dione in culture media at 37 °C for 30 min. For assays, the media were replaced with Dulbecco's phosphate buffered saline (D-PBS) containing reactive oxygen species sensitive fluorophore 6-carboxy-2′,7′-dichloro-dihydro-fluorescin diacetate (CH_2DCF-DA, 5 μM) and incubated for 15 min at 37 °C.

3. Results

3.1. Effect of pretreatment with prazosin, propranolol or a cyclooxygenase inhibitor, indomethacin, on 5-HT_{2A} receptor-mediated increased vascular resistance

A possible contribution of α-, or β-adrenoceptors to 5-HT_{2A} receptor-mediated increased vascular resistance was tested by pretreatment with an α-adrenoceptor antagonist, prazosin (0.1 mg/kg), or a β-adrenoceptor antagonist, propranolol (2 mg/kg). The α-methyl-serotonin-mediated acute vasoconstrictor effect was not affected by the α-, or β-adrenoceptor blocking agents (Fig. 1). In addition, pretreatment with indomethacin (2 mg/kg) did not affect the α-methyl-serotonin-mediated acute vasoconstrictor effect (Fig. 1). This observation is consistent with the previous report that 5-HT acted independently of the prostaglandin system (Ding et al., 1989).

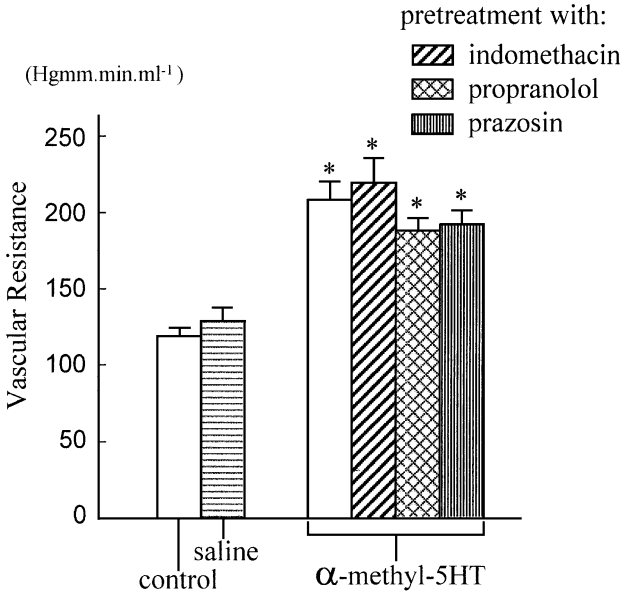

Fig. 1. Effect of α-methyl-serotonin on vascular resistance in presence or absence of indomethacin, prazosin or propranolol. Indomethacin (2 mg/kg, i.p.), prazosin (0.1 mg/kg, i.p.) or propranolol (2 mg/kg, i.p.) was administered intraperitoneally 30 min before α-methyl-serotonin (2 mg/kg, i.p.) administration. Vascular resistance in the hindlimb was calculated as mean arterial blood pressure in millimeters of mercury divided by femoral blood flow in milliliters per minute. At 40–45 min after α-methyl-serotonin administration, femoral blood flow and arterial pressure were recorded. Each value is the mean ± S.E. ($n=4-6$). $*p<0.05$, compared with the values before administration. α-MHT, α-methyl-serotonin.

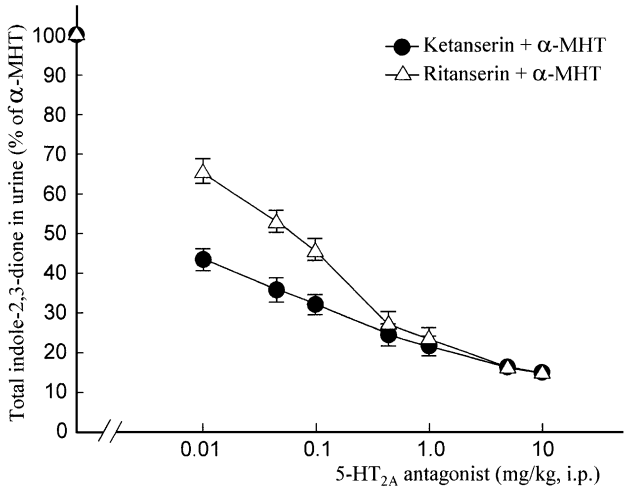

Fig. 2. Dose-dependent inhibition of α-methyl-serotonin-induced indole-2,3-dione production by 5-HT_2 antagonists. Ketanserin (0.01–5 mg/kg, i.p.) or ritanserin (0.01–5 mg/kg, i.p.) was administered 30 min before α-methyl-serotonin (2 mg/k, i.p.) administration. Each value is the mean ± S.E.M. (n = 4–6). α-MHT, α-methyl-serotonin.

3.2. Effect of 5-HT_{2A} receptor antagonists on α-methyl-serotonin-induced indole-2,3-dione formation

Pretreatment with the 5-HT_{2A} receptor antagonists ketanserin (0.01, 0.1, 1.5 mg/kg) or ritanserin (0.01, 0.1, 1.5 mg/kg), caused a dose-dependent inhibition of indole-2,3-dione formation elicited by α-methyl-serotonin (Fig. 2). The ability of α-methyl-serotonin to prompt indole-2,3-dione formation can be ascribed to specific 5-HT_{2A} receptor stimulation in the periphery.

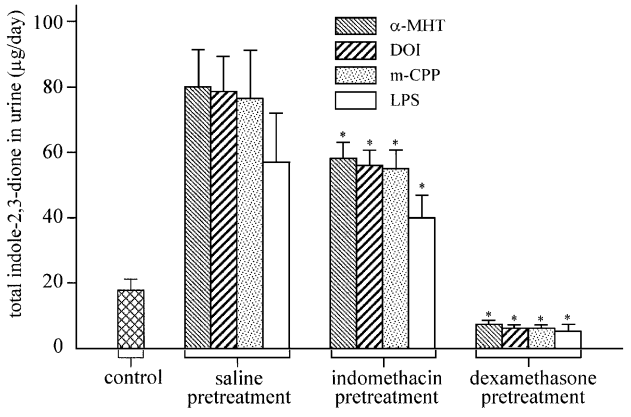

Fig. 3. Effects of pretreatment with either dexamethasone (2.0 mg/kg/day, i.p.) or indomethacin (2.0 mg/kg, i.p.) on the increases in indole-2,3-dione elicited by lipopolysaccharide or 5-HT_{2A} receptor agonists, α-methyl-serotonin, *m*-CPP and DOI. Dexamethasone or indomethacin was given as pretreatment 3–8 h before lipopolysaccharide or 5-HT_{2A} receptor agonists administration. Values show total indole-2,3-dione in rat urine for 24 h after the injections of α-methyl-serotonin (2.0 mg/kg, i.p.), *m*-CPP (0.1 mg/kg, i.p.) and DOI (0.1 mg/k, i.p.), with exception of lipopolysaccharide (0.05 mg/kg, i.p.) with the values for 48–72 h. Each value is the mean ± S.E. (n = 4). * p < 0.05, compared with the saline-pretreated group at 24 or 72 h (in lipopolysaccharide) (Student's t-test). α-MHT, α-methyl-serotonin.

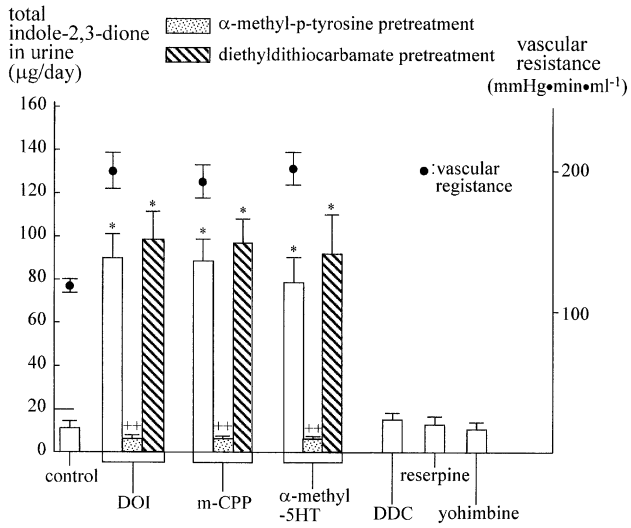

Fig. 4. Effect of α-methyl-*p*-tyrosine (300 mg/kg, i.p.) or diethyldithiocarbamate (500 mg/kg, i.p.) pretreatment on 5-HT_{2A} receptor agonist-induced increases in indole-2,3-dione, shown with effects of reserpine (5 mg/kg, i.p.), yohimbine (5 mg/kg, i.p.) and diethyldithiocarbamate (500 mg/kg, i.p.) on indole-2,3-dione levels. α-methyl-*p*-tyrosine or diethyldithiocarbamate (500 mg/kg, i.p.) was given as pretreatment 2 h before the 5-HT_{2A} receptor agonist injections. Values show total indole-2,3-dione in rat urine for 24 h after final injection. Each value is the mean ± S.E. of four to six determinations in each group. * p < 0.01, compared with the saline-pretreated group at 24 h (Student's t-test). ^{++}p < 0.01, compared with the values for group treated with 5-HT_{2A} agonist alone. α-MT, α-methyl-*p*-tyrosine.

3.3. Effect of anti-inflammatory agents, dexamethasone and indomethacin, on α-methyl-serotonin-induced indole-2,3-dione formation

Pretreatment with dexamethasone (2 mg/kg/day) or indomethacin (2 mg/kg) suppressed the α-methyl-serotonin-induced increase in indole-2,3-dione (Fig. 3). The difference in ability to suppress indole-2,3-dione formation between two anti-inflammatory agents may indicate the possible involvement of activation of nitric oxide synthase in indole-2,3-dione generation. The inhibitory action of anti-

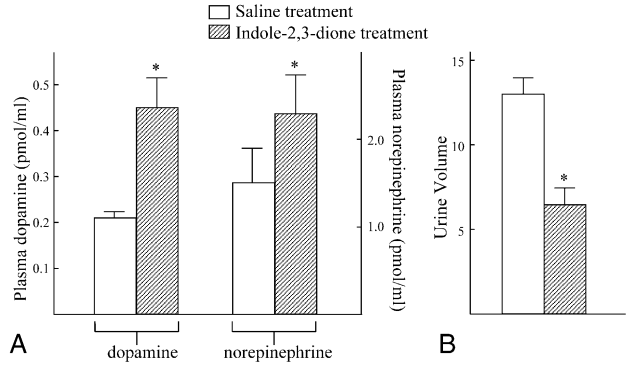

Fig. 5. Effect of exogenous indole-2,3-dione on plasma dopamine, norepinephrine and urine volume. (A) Plasma catecholamine levels were measured 2 h after indole-2,3-dione (100 mg/kg, i.p.) treatment. (B) Urine volume was measured for 24 h of indole-2,3-dione treatment. Each value is the mean ± S.E. (n = 6). * p < 0.05, compared with the saline-treated group.

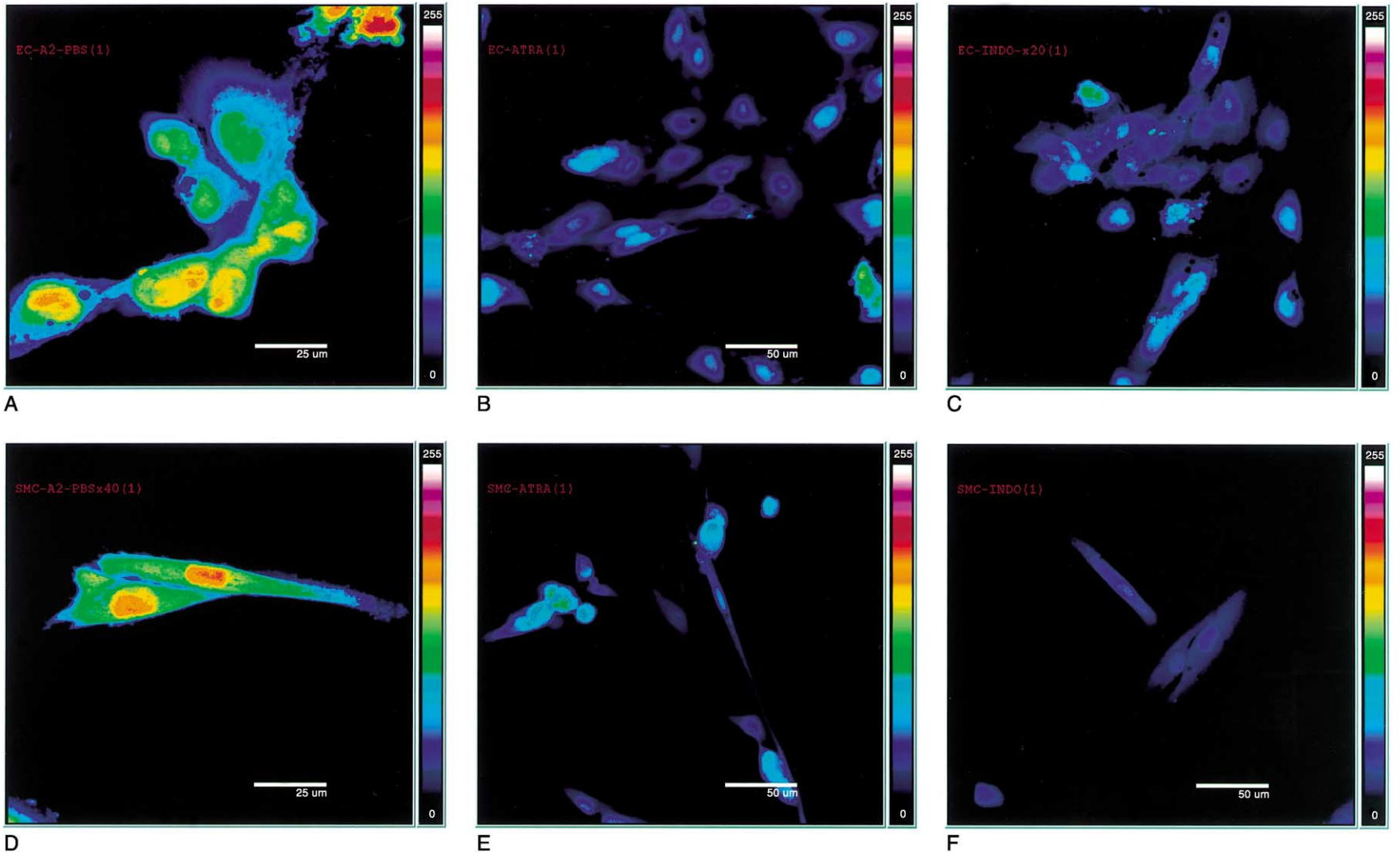

Fig. 6. Effect of indole-2,3-dione (20 μM) pretreatment on angiotensin AT_1-mediated reactive oxygen species generation in human umbilical vein endothelial cells (A–C) and smooth muscle cells (D–F). Angiotensin II (10^{-7} M)-enhanced reactive oxygen species generation (A,C) after incubation for 30 min was abolished by pretreatment with either angiotensin AT_1 receptor antagonist, TCV116 (10^{-7} M) (B,E), or indole-2,3-dione (C,F).

inflammatory agents on indole-2,3-dione production was exerted on the lipopolysaccharide-induced increase in indole-2,3-dione at 72 h.

3.4. Effect of a tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine, and a dopamine β hydroxylase inhibitor, diethyldithiocarbamate, on 5-HT$_{2A}$ receptor-mediated increase in indole-2,3-dione

Tyrosine hydroxylase and dopamine β hydroxylase are two key enzymes of catecholamine biosynthesis. Tyrosine hydroxylase catalyzes the initial and rate-limiting step of catecholamine biosynthesis, Tyr hydroxylation, although it also catalyzes Phe hydroxylation. Dopamine β hydroxylase is detected on vesicular membranes and is also released by exocytosis. It catalyzes hydroxylation of dopamine to form norepinephrine. In this study, α-methyl-*p*-tyrosine (300 mg/kg) completely abolished the increase in indole-2,3-dione elicited by 5-HT$_{2A}$ receptor agonists, whereas diethyldithiocarbamate (500 mg/kg) tended to increase indole-2,3-dione (Fig. 4).

3.5. Effect of an α2-adrenoceptor antagonist, yohimbine, a vesicular transporter inhibitor, reserpine, and diethyldithiocarbamate on indole-2,3-dione production

Yohimbine has been reported not to alter DOPA levels despite its potentiating effect on norepinephrine release and turnover. Reserpine, often used to deplete norepinephrine in sympathetic nerve cells, has also been also described not to decrease DOPA significantly (Fisher et al., 2000). In vivo studies have shown that norepinephrine, but not dopamine, is drastically reduced by diethyldithiocarbamate (Bloom et al., 1977). The present observation that neither yohimbine (5 mg/kg), reserpine (5 mg/kg) nor diethyldithiocarbamate altered indole-2,3-dione formation (Fig. 4) is in agreement with the above mentioned result of the discrepancy between the effects of α-methyl-*p*-tyrosine and diethyldithiocarbamate on indole-2,3-dione formation.

3.6. Effect of exogenous indole-2,3-dione on plasma catecholamine concentrations and urine volume

Indole-2,3-dione (100 mg/kg, i.p.) administration induced a significant increase in plasma dopamine and norepinephrine levels and a significant decrease in 24-h urine volume (Fig. 5A,B). The ability of exogenous indole-2,3-dione to penetrate into tissues and organs has been established.

3.7. Effect of indole-2,3-dione on angiotensin AT$_1$-mediated reactive oxygen species generation in human umbilical vein endothelial cells and smooth muscle cells

The angiotensin II (10^{-7} M)-enhanced generation of reactive oxygen species (a,c) after incubation for 30 min was abolished by pretreatment with either the angiotensin AT$_1$ receptor antagonist, TCV116 (Takeda Pharmaceutical) (10^{-7} M) (b,e), or indole-2,3-dione (20 μM) (c,f) (Fig. 6A–E).

4. Discussion

Indole-2,3-dione formation was now shown to be induced specifically by 5-HT$_{2A}$ receptor-mediated inflammatory process. All aspects of the data support the conclusion that 5-HT$_{2A}$ receptor-mediated decreased capillary flow alone could cause the inflammatory process and resultant formation of indole-2,3-dione and that DOPA/dopamine, not norepinephrine, is the agent mediating the development and progression of inflammation triggered by an insufficient oxygen supply due to decreased capillary flow.

First, indomethacin could not modify the peripheral 5-HT$_{2A}$ receptor-mediated increased vascular resistance but suppressed, as well as did dexamethasone, the 5-HT$_{2A}$ receptor agonists-induced increases in indole-2,3-dione. An acute hemodynamic change elicited by 5-HT$_{2A}$ receptor agonists occurred independently of the prostaglandin system and caused an inflammatory process involving the prostaglandins. Second, conditions that increase DOPA/dopamine levels alone led to a drastic increase in indole-2,3-dione formation. Neither conditions that prompt, or cause to deteriorate, the release of catecholamines from sympathetic nerve terminals nor dopamine β hydroxylase inhibition led to a significant change in indole-2,3-dione formation, whereas tyrosine hydroxylase inhibition resulted in the complete abolition of 5-HT$_{2A}$ receptor agonists-induced increases in indole-2,3-dione. Third, a possible involvement of the 5-HT$_2$ receptor agonists-induced release of norepinephrine from sympathetic nerve terminals in the prompting of indole-2,3-dione formation can be eliminated. Neither α-adrenoceptor blockade with prazosin nor β-adrenoceptor blockade with propranolol had a significant effect on α-methyl-serotonin-elicited vasoconstriction. Thus, the efficacy of TH inhibition to abolish α-methyl-serotonin-induced indole-2,3-dione formation is exclusively ascribable to depletion of DOPA/dopamine. Two possibilities arise that DOPA/dopamine plays a crucial role in the progression of inflammation and that either Phe, Tyr, DOPA or dopamine yields a precursor of indole-2,3-dione.

The chemical properties of DOPA and dopamine make them strong candidates for endogenous cytotoxicants, which would cause both non-neuronal and neuronal cell death. The pathological relevance to inflammatory disease states of DOPA/dopamine-derived quinones generated through non-enzymatic oxidation (Graham, 1978; Graham et al., 1978; Klegeris et al., 1995; Basma et al., 1995) and enzymatic conversion by tyrosinase has been well documented, whereas hydrogen peroxide and other reactive oxygen species downstream of the peroxide (Halliwell, 1992) yielded by either monoamine oxidase-catalyzed dopamine metabolism (Pizzinat et al., 1999) or autooxidation

(Graham et al., 1978) have also been widely seen as participants in inflammatory disease states. The fact that antioxidants and reducing agents prevent quinone formation from DOPA/dopamine and reduce those quinones back to DOPA/dopamine (Hastings and Zigmond, 1994; Graham et al., 1978; Graham, 1978) suggests strongly that the initial oxidizing condition (increased reactive oxygen species) is a crucial trigger for quinone formation. It is well known that conditions that lead to cytotoxic effects of dopamine include ischemia/hypoxia.

DOPA/dopamine-quinones are well known to modify covalently nucleophilic groups of free amino acids or amino acyl residues in proteins. Cysteinyl-catechols formed via DOPA/dopamine-derived reactive quinones are known to cause enzyme inhibition with respect to neuronal toxicity (Li et al., 1998). Identification of amino acyl-catechols and their oxidative metabolites awaits further study. The observation in our preliminary study that co-administration of Phe and Tyr enhanced indole-2,3-dione formation significantly (data not shown) may make Phe-DOPA/dopamine quinone a strong candidate precursor for indole-2,3-dione, considering it has a non-hydroxylated benzene ring.

Evidence has been accumulating that plasma DOPA, but not plasma norepinephrine, reflects sympathetic activity (Kvetnansky et al., 1992; Floras et al., 1986). It is known that DOPA inhibits DOPA decarboxylase (aromatic amino acid decarboxylase) (Schott and Clark, 1952; Tate et al., 1971). In this context, the previous observation that indole-2,3-dione was significantly increased during stress (Tozawa et al., 1998) is consistent with the present results.

The possibility cannot be eliminated that peripheral 5-HT_{2A} receptor-mediated indole-2,3-dione formation reflects an intrarenal inflammatory process because 5-HT_{2A} receptor stimulation can produce flow-limiting spasm in the renal beds and resultant local ischemia.

The results obtained suggest a potential sympathoexcitatory role for exogenous indole-2,3-dione, which would be evident in conditions characterized by decreased capillary flow and oxygen supply. The sympathoexcitatory action of indole-2,3-dione may be attributed to inhibition of sympathoinhibitory ANP signaling. Given the fact that the microcirculation is the site of indole-2,3-dione formation, local indole-2,3-dione concentrations would be much higher than those reported previously (range 0.02–11.0 μg/g) (Watkins et al., 1990) for whole organ homogenates.

In the present study, indole-2,3-dione abolished the angiotensin AT_1 receptor-mediated intracellular generation of reactive oxygen species (Griendling et al., 1997) in both human umbilical vein endothelial cells and smooth muscle cells at 20 μM (Fig. 6), though it remains to be elucidated whether it inhibits NADPH oxidase itself or not. Together with the fact that indole-2,3-dione also inhibits monoamine oxidase (Glover et al., 1988), the anticipated sulfhydryl reactivity of indole-2,3-dione (Fig. 7) may make it a contributing factor to regulation of either NAD(P)H oxidizing flavoenzyme activities or conversion of dehydrogenases to reactive oxygen species-generating oxidases (McKelvey et al., 1988). Electrophilic indole-2,3-dione may evoke inhibition of mitochondrial complex I activity and irreversible loss of glutathione.

In conclusion, a peripheral 5-HT_{2A} receptor-mediated increase in vascular resistance prompted the formation of a highly oxidized amino acid, indole-2,3-dione, an endogenous ANP signaling inhibitor, through inflammation. A decreased capillary flow alone may be a crucial trigger for the initiation of inflammation. Whether DOPA and/or dopamine contributes to the progression of the inflammatory process or the generation of a precursor of indole-2,3-dione awaits further studies. With respect to a possible pathophysiological role, the sympathoexcitatory action of indole-2,3-dione suggests that 5-HT_{2A} receptor stimulation may cause peripheral sympathetic overactivity via ANP signaling inhibition. The sulfhydryl reactivity of indole-2,3-dione may make it a regulator of mitochondrial function and cellular redox states via enzyme inhibition and/or dehydrogenase–oxidase conversion.

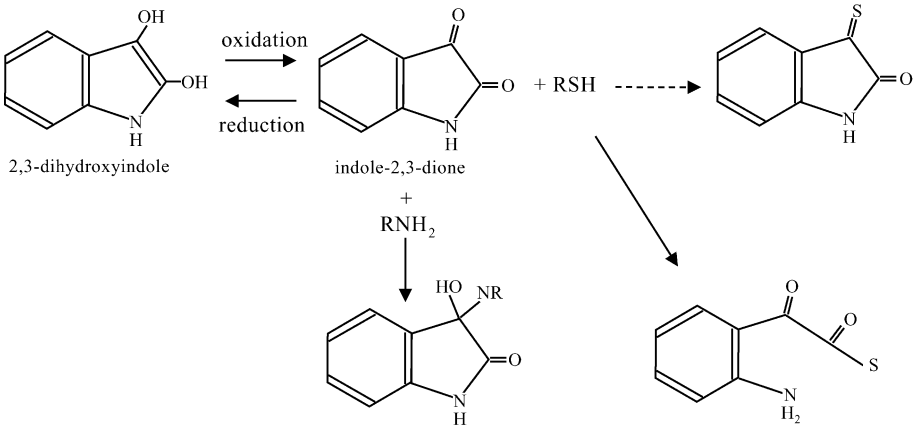

Fig. 7. Possible conversion between two oxidative metabolites indole-2,3-dione and 2,3-dihydroxyindole, shown with possible sulfhydryl reactivity.

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