The Cytochrome P450 Inhibitor Ketoconazole Potentiates 5-Hydroxytryptamine-Induced Contraction in Rat Aorta

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ABSTRACT

5-Hydroxytryptamine (5-HT; serotonin) is a potent vasoconstrictor and smooth muscle mitogen. Substances that produce similar responses also stimulate production of superoxide. We sought to determine whether 5-HT stimulates production of superoxide. 5-HT can be metabolized by cytochrome P450 to nitric oxide (NO), which scavenges superoxide. Thus, we hypothesized that inhibiting cytochrome P450 would potentiate 5-HT-induced contraction and reveal 5-HT-stimulated superoxide. In isolated tissue bath experiments using endothelium-intact rat aorta, the cytochrome P450 inhibitor ketoconazole (KTZ; 1–50 μM) caused a maximum 8-fold leftward shift in the 5-HT concentration-response curve that was not observed when aorta were stimulated with phenylephrine or KCl. 5-HT did not stimulate concentration-dependent increases in superoxide levels as measured by a lucigenin-enhanced chemiluminescent superoxide assay. KTZ (10 μM) did not reveal 5-HT-stimulated superoxide. The NO inhibitor Nω-nitro-L-arginine (L-NNA) (100 μM) with or without KTZ (10 μM) potentiated 5-HT-induced contraction independently of NADPH oxidase-derived superoxide but also did not reveal 5-HT-stimulated superoxide. Metabolism of 5-HT to NO depends on catalase, but the catalase inhibitor 3-amino-1,2,4-triazole (50 mM) attenuated 5-HT-induced contraction. Removal of endothelium did not alter the effects of KTZ on 5-HT-induced contraction, and, in endothelium-intact aorta, KTZ did not decrease acetylcholine-induced relaxation. Unlike KTZ, the cytochrome P450 inhibitor 1-aminobenzotriazole (0.5 mM) and clotrimazole (10 μM) did not potentiate 5-HT-induced contraction. Moreover, 14,15-epoxyeicosa-5(Z)-enoic acid (10 μM), an epoxyeicosatrienonic acid antagonist, caused a small rightward shift in the 5-HT concentration-response curve. These data suggest KTZ acts by a potentially novel mechanism to potentiate 5-HT-induced contraction.

5-Hydroxytryptamine (5-HT; serotonin) stimulates several physiological responses in vascular smooth muscle, most notably cell growth (Nemecek et al., 1986) and vasoconstriction. In animals with normal blood pressure, both of these responses are mediated by the 5-HT2A receptor, although by somewhat independent mechanisms (Florian and Watts, 1998). Reactive oxygen species (ROS), particularly superoxide (O2–), and hydrogen peroxide (H2O2), mediate vascular smooth muscle cell (VSMC) growth and vasoconstriction, similar to 5-HT. Angiotensin II and endothelin-1, as angiotensin II and endothelin-1 that stimulate VSMC growth and vasoconstriction, similar to 5-HT, induce ROS production in arteries (Griendling et al., 1994; Duerrschmidt et al., 2000), and this is a mechanism thought to contribute to the pathology of vascular dysfunction in hypertension (Touyz and Schiffrin, 2004). Establishing whether 5-HT can stimulate arterial O2– production was the original impetus for this study.

In arteries, as well as neurons, 5-HT is taken up into VSMCs by the 5-HT transporter, and it is primarily metabolized by monoamine oxidase A into 5-hydroxyindoleacetic acid (5-hydroxyindole acetic acid; Ni and Watts, 2006). However, in hepatocytes, 5-HT can be metabolized by cytochrome P450, specifically the CYP2B6, -2C9, and -2C19 isoforms, as well as angiotensin II and endothelin-1 that stimulate VSMC growth and vasoconstriction, similar to 5-HT, induce ROS production in arteries (Griendling et al., 1994; Duerrschmidt et al., 2000), and this is a mechanism thought to contribute to the pathology of vascular dysfunction in hypertension (Touyz and Schiffrin, 2004). Establishing whether 5-HT can stimulate arterial O2– production was the original impetus for this study.

In arteries, as well as neurons, 5-HT is taken up into VSMCs by the 5-HT transporter, and it is primarily metabolized by monoamine oxidase A into 5-hydroxyindoleacetic acid (5-hydroxyindole acetic acid; Ni and Watts, 2006). However, in hepatocytes, 5-HT can be metabolized by cytochrome P450, specifically the CYP2B6, -2C9, and -2C19 isoforms, producing hydroxylamine (Fradette et al., 2004). In the presence of H2O2 and catalase, this hydroxylamine is converted into nitric oxide (NO). If a similar metabolism of 5-HT to NO occurred in arteries, the NO could elicit vasorelaxation.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); ROS, reactive oxygen species; O2–, superoxide; VSMC, vascular smooth muscle cell; EET, epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; KTZ, ketoconazole; PE, phenylephrine; ACh, acetylcholine; L-NNA, Nω-nitro-L-arginine; 3-AT, 3-amino-1,2,4-triazole; ABT, 1-aminobenzotriazole; 14,15 EEZE, 14,15-epoxyeicosa-5(Z)-enoic acid; JKB, Jude’s Kreb; DDC, diethyldithiocarbamic acid; NOS, nitric-oxide synthase.
Moreover, since O$_2^-$ reacts rapidly with NO to produce peroxynitrite (OONO$^-$), NO could efficiently scavenge and deplete O$_2^-$, making the O$_2^-$ stimulated by 5-HT difficult to measure.

Cytochrome P450 enzymes can contribute to arterial tone by production of vasoactive substances other than NO. Cytochrome P450 enzymes catalyze the formation of epoxygenosatrienoic acids (EETs) and 20-hydroxysaferatenoic acid (20-HETE), metabolites of arachidonic acid (Roman, 2002). EETs are putative endothelium-derived hyperpolarizing factors that cause relaxation of blood vessels by activating K$^+$ channels on VSMCs (Roman, 2002). In contrast, 20-HETE is a potent vasoconstrictor, causing depolarization of VSMCs by blocking calcium-activated K$^+$ channels (Roman, 2002).

We hypothesized that 5-HT stimulates O$_2^-$ production in rat aorta through the 5-HT$_2A$ receptor. We first had to determine whether 5-HT was simultaneously metabolized by cytochrome P450, producing NO. Furthermore, we predicted that if this was the case, NO would scavenge the O$_2^-$.

We used ketoconazole (KTZ) as a pharmacological inhibitor of cytochrome P450. KTZ inhibits cytochrome P450 by binding to the cytochrome component of the monoxygenasen complex (Sheets and Mason, 1984), interfering with cytochrome-mediated oxidation (Meredith et al., 1985). KTZ most potent affects the activity of the CYP3A family (Maurice et al., 1992; Olkkola et al., 1994), but KTZ can inhibit other isofoms of cytochrome P450, including CYP2B6, -2C9, and -2C19 (Stresser et al., 2004). We predicted that KTZ would prevent biotransformation of 5-HT to NO, thus revealing O$_2^-$ stimulated by 5-HT. Moreover, since NO is a relaxant in arteries, we expected that KTZ should potentiate 5-HT-induced contraction. Although we did not reveal cytochrome P450 metabolism of 5-HT to NO or 5-HT-stimulated superoxide production, we discovered a potentially novel mechanism by which KTZ alters 5-HT contraction.

Materials and Methods

Male Sprague-Dawley rats (0.300–0.350 kg; Charles River Breeding Laboratories, Portage, MI) were used for all experiments. All animal procedures were performed in accordance with the guidelines of the Michigan State University All-University Committee on Animal Use and Care.

Isolated Tissue Bath Protocol. Rats were deeply anesthetized (60 mg/kg pentobarbital i.p.), and thoracic aorta was removed and placed in physiological salt solution, pH 7.2, containing 103 mM NaCl, 4.7 mM KCl, 1.18 mM KH$_2$PO$_4$, 1.17 mM MgSO$_4$·7H$_2$O, 1.6 mM CaCl$_2$·2H$_2$O, 14.9 mM NaHCO$_3$, 5.5 mM dextrose, and 0.03 mM CaNa$_2$EDTA. Aortae were cleaned of excess fat and connective tissue. Tissues were contracted with a half-maximal concentration of PE (10$^{-6}$ M), and acetylcholine (ACh; 1 $\mu$M) was added. Endothelial removal was verified by a $>20\%$ relaxation to ACh. Tissues were again washed until baseline was reached, and then they were incubated for 1 h with KTZ (10 $\mu$M), N$^\omega$-nitro-l-arginine (l-NNA; 100 $\mu$M), apocynin (100 $\mu$M), 3-amino-1,2,4-triazole (3-AT; 50 $\mu$M), 1-aminobenzotriazole (ABT; 0.5 $\mu$M), clotrimazole (10 $\mu$M), 14,15-epoxyeicosatetraenoic acid (ETE; 10 $\mu$M), or vehicle (dimethyl sulfoxide for KTZ and clotrimazole; distilled water for l-NNA, apocynin, 3-AT, and ABT; ethanol for 14,15-ETE). A cumulative concentration-response curve to 5-HT (1 nM–0.3 mM), PE (1 nM–0.3 mM), KCl (10–100 $\mu$M), ACh (1 nM–0.3 $\mu$M), or o-methyl-5-HT (1 nM–0.3 $\mu$M) was performed.

Superoxide Assay Using Lucigenin-Enhanced Chemiluminescence. Endothelium-intact thoracic aortae were removed from deeply anesthetized rats (pentobarbital; 60 mg/kg i.p.), and they were placed in chilled Krebs-HEPES buffer (KJB; pH 7.4) containing 20 mM HEPES, 11.9 mM NaCl, 0.46 mM KCl, 0.10 mM MgSO$_4$·7H$_2$O, 0.015 Na$_2$HPO$_4$, 0.04 KH$_2$PO$_4$, 0.5 NaHCO$_3$, 1.2 CaCl$_2$, and 5.5 dextrose. Excess fat and adventitial tissue were removed, and aortae were cut into rings (>5 $\mu$g wet weight). Aortae were incubated in JKB with KTZ (10 $\mu$M), l-NNA (100 $\mu$M), apocynin (100 $\mu$M), or vehicle (dimethyl sulfoxide for KTZ and water for l-NNA and apocynin), and they were equilibrated for 30 min at 37°C. Diethyldithiocarbamic acid (DDC; 10 mM), an inhibitor of superoxide dismutase, and 5-HT (10 nM–10 $\mu$M) were added, and aortae were incubated for 4 h. Aortae were incubated with fresh JKB with lucigenin (5 $\mu$M) for 10 min, and chemiluminescence was measured using a luminometer (TD 20/20 luminometer; Turner Designs, Sunnyvale, CA). Ten chemiluminescence measurements, each integrated over 30 s, were taken and the O$_2^-$ scavenger 4,5-dihydroxy-1,3-dibenzenedisulfonic acid disodium salt (tiron; 10 $\mu$M) was added to aortae. After a 10-min incubation, 10 chemiluminescence measurements were made. Aortae were then blotted dry and weighed. The O$_2^-$ signal was taken to be the difference of the average luminescence before and after tiron and O$_2^-$ levels (nanomoles per minute per milligram) were calculated using a cytochrome c oxidase standard curve.

Data Analysis. Data are presented as mean values ± S.E.M. for the number of animals indicated in parentheses. Data from isolated tissue bath experiments are presented as a percentage of the initial PE (10 $\mu$M) contraction. Agonist EC$_{50}$ values were calculated using a nonlinear regression analysis with the algorithm (effect = maximum response/1 + EC$_{50}$/agonist concentration) and the computer program Prism (GraphPad Software Inc., San Diego, CA). When two groups were compared, the appropriate Student’s t test was used. When comparing three or more groups, analysis of variance followed by Bonferroni’s post hoc test was performed. A p value $<$0.05 was considered statistically significant.

Chemicals. Solutions of compounds were prepared in the appropriate vehicle as indicated. All agonists were solubilized in water. 5-HT, PE, ACh, KTZ, l-NNA, 3-AT, ABT, clotrimazole, apocynin, DDC, tiron, and lucigenin were purchased from Sigma-Aldrich (St. Louis, MO), and α-methyl-5-HT was purchased from Sigma/RBI (Natick, MA). 14,15-ETE was synthesized within the laboratory of John Falck (The University of Texas Southwestern Medical Center, Dallas, TX).

Results

KTZ Potentiates 5-HT-Induced Contraction in Rat Aorta. The cytochrome P450 3A4 inhibitor KTZ caused a leftward shift in the 5-HT concentration-response curve in endothelium-intact rat at concentrations as low as 1 $\mu$M (Fig. 1A; Table 1). To test whether KTZ specifically affects 5-HT-induced contraction, we incubated aorta with the maximal concentration of KTZ (50 $\mu$M), and we stimulated with two different contractile stimuli: PE, an $\alpha_2$-adrenergic agonist, and KCl, an activator of L-type Ca$^{2+}$ channels. A maximal concentration of KTZ did not cause a shift in the PE (Fig. 1B) or KCl (Fig. 1C) concentration-response curves.
KTZ Does Not Reveal 5-HT-Stimulated $O_2^\cdot$ To determine whether 5-HT can induce production of $O_2^\cdot$ in rat aorta, we measured $O_2^\cdot$ levels in rat aorta stimulated with 5-HT using lucigenin-enhanced chemiluminescence. 5-HT (10 nM–10 μM), incubated for 4 h, did not stimulate a concentration-dependent increase in superoxide levels (Fig. 2A) in endothelium-intact rat aorta. Preliminary experiments showed shorter 5-HT incubation (5–180 min) did not capture a superoxide signal and that addition of the monoamine oxidase inhibitor pargyline did not amplify the signal. Importantly, the concentrations of 5-HT used elicited potent vasoconstriction (Fig. 1A). We predicted that the 5-HT-stimulated $O_2^\cdot$ was being depleted by NO produced from cytochrome P450-mediated metabolism of 5-HT. If so, inhibition of cytochrome P450 should prevent NO production and thus expose 5-HT-stimulated $O_2^\cdot$. However, KTZ (10 μM) did not reveal 5-HT-induced $O_2^\cdot$ (Fig. 2A). In these experiments, angiotensin II (100 nM) was used as a positive control. To examine the effects of other sources of NO on 5-HT-stimulated $O_2^\cdot$, we stimulated aorta with 5-HT (10 nM–10 μM) in the presence of the nonselective NOS inhibitor L-NNA (100 μM). L-NNA did not reveal 5-HT-stimulated $O_2^\cdot$, similar to KTZ (Fig. 2A), suggesting that endogenous levels of NO do not quench normal levels of $O_2^\cdot$. To determine whether both cytochrome P450- and NOS-derived NO contributed to depletion of endogenous $O_2^\cdot$, we treated aorta with both KTZ (10 μM) and L-NNA (100 μM). KTZ and L-NNA together did not change 5-HT-stimulated $O_2^\cdot$ levels (Fig. 2A). Despite not revealing 5-HT-stimulated $O_2^\cdot$, L-NNA caused a 9-fold leftward shift in the 5-HT concentration-response curve (Fig. 2B; Table 1). In the presence of L-NNA, KTZ further potentiated 5-HT-induced contraction (Fig. 2B; Table 1).

NADPH Oxidase-Derived $O_2^\cdot$ Does Not Explain Potentiation of 5-HT-Induced Contraction by KTZ and L-NNA. The results of Fig. 2 suggest that the enhanced contraction of aorta to 5-HT caused by L-NNA and KTZ was not due to increased $O_2^\cdot$ levels. Further supporting this conclusion, the NADPH oxidase inhibitor apocynin (100 μM) together with KTZ (10 μM) and L-NNA (100 μM) did not alter 5-HT-stimulated $O_2^\cdot$ levels (Fig. 3A). In addition, apocynin did not reduce the potentiation of 5-HT-induced contraction caused by KTZ and L-NNA (Fig. 3B; Table 1). In both contractile and chemiluminescence experiments, apocynin (100 μM) did not reveal 5-HT-stimulated $O_2^\cdot$. Therefore, KTZ does not reveal 5-HT-stimulated $O_2^\cdot$, and the enhanced contraction of aorta to 5-HT caused by L-NNA and KTZ is not due to increased $O_2^\cdot$ levels.
alone did not reveal 5-HT-stimulated O₂*, and since metabolism of 5-HT was reported to be dependent on catalase, we examined the effects of the catalase inhibitor 3-AT on 5-HT-induced contraction. In contrast to KTZ, 3-AT (50 mM) did not significantly shift the 5-HT concentration-response curve [vehicle EC₅₀ (-log M) = 5.6 ± 0.1; 3-AT EC₅₀ (-log M) = 5.6 ± 0.1], and it inhibited the maximal contraction elicited by 5-HT in endothelium-intact rat aorta (Fig. 4).

Effects of KTZ Are Independent of Endothelium and NOS. Since catalase did not potentiate 5-HT-induced contraction in these vessels, it is unlikely that cytochrome P450 metabolizes 5-HT to NO in the manner describe by Fradette et al. (2004). However, since KTZ and l-NNA similarly potentiated 5-HT-induced contraction, we tested the ability of KTZ to inhibit endothelial NOS (NOS3), the major source of NOS in arteries. The effects of KTZ were independent of endothelium, because KTZ (10 μM) caused similar potentiation of 5-HT-induced contraction in aorta denuded of endothelium compared with endothelium-intact aorta (Fig. 5A; Table 2). KTZ (10 μM) did not reduce ACh-induced relaxation of endothelium-intact aorta contracted with the half-maximal concentration of PE (Fig. 5B). Since other isoforms of NOS, specifically NOS1, are present in vascular smooth muscle (Brophy et al., 2000), we tested the effects of l-NNA on 5-HT-induced contraction in aorta denuded of endothelium.
L-NNA (100 μM) did not shift the 5-HT concentration-response curve in these vessels (Fig. 5C; Table 2).

**Effects of KTZ Are Not Cytochrome P450-Specific.** We next examined whether effects of KTZ could be observed with another cytochrome P450 inhibitor. Unlike KTZ, the general cytochrome P450 inhibitor ABT (0.5 mM) did not enhance 5-HT-induced contraction, but it reduced the maximal contraction elicited by 5-HT in endothelium-denuded aorta (Fig. 6A; Table 2). Similarly, clotrimazole (10 μM), an imidazole P450 inhibitor, did not potentiate 5-HT-induced contraction of endothelium-denuded rat aorta (Fig. 6B; Table 2). The concentrations of inhibitors used were based on published literature (Emoto et al., 2003; He et al., 2003; Polsky-Fisher et al., 2006, Randriamboavonjy et al., 2005).

**KTZ Does Not Inhibit Production of EETs.** Since the potentiation of 5-HT-induced contraction caused by KTZ is not likely due to inhibition of cytochrome P450-mediated metabolism of 5-HT to NO, we examined other possible mechanisms. The production of EETs, putative endothelium-derived hyperpolarizing factors, from arachidonic acid is catalyzed by cytochromes P450. However, the EET antagonist 14,15-EEZE (10 μM) inhibited, rather than enhanced, 5-HT-induced contraction of endothelium-denuded rat aorta (Fig. 7; Table 2).

**Effects of KTZ Are 5-HT₂A Receptor-Dependent.** Finally, to determine whether the effects of KTZ are dependent on 5-HT₄ receptor activation, we stimulated KTZ-exposed endothelium-denuded aorta with the 5-HT₂ receptor agonist α-methyl-5-HT (Fig. 8). KTZ (10 μM) potentiated α-methyl 5-HT-induced contraction [vehicle EC₅₀ (−log M) = 6.38 ± 0.07; KTZ EC₅₀ (−log M) = 6.60 ± 0.06].

**Discussion**

**5-HT and O₂⁻.** In the cardiovascular system, 5-HT is a potent vasoconstrictor and smooth muscle mitogen (Nemecek et al., 1986), but the signal transduction pathways for both responses are somewhat independent (Florian and Watts, 1998). In rat aorta, 5-HT stimulates vasoconstriction by activating the 5-HT₂A receptor (Cohen et al., 1981), a G protein-coupled receptor classically linked to phospholipase C and L-type calcium channels by G₉/₁₁ (Roth et al., 1998). Other agonists of G protein-coupled receptors that stimulate vasodilation and smooth muscle cell growth, such as angiotensin II and endothelin-1, stimulate production of ROS, including O₂⁻ and H₂O₂ (Griendling et al., 1994; Duersschmidt et al., 2000). Since ROS can mediate both vasodilation and vascular smooth muscle growth (Rao and Berk, 1992; Griendling et al., 1994, 2000; Touyz and Schiffrin, 2004), they are excellent candidates for mediating these responses when elicited by 5-HT.

The current study was undertaken to determine whether 5-HT stimulates production of ROS, specifically O₂⁻, in rat aorta. We were unable to measure 5-HT-stimulated produc-
interaction would be the decreased bioavailability of NO, which would lead to impaired relaxation. Overall, our data refute this hypothesis. We tried multiple times of incubation and inhibition of 5-HT metabolism in the lucigenin assay, but none of these manipulations revealed 5-HT stimulated O$_2^-$ production. One other possible explanation for our negative findings was the inclusion of DDC as a superoxide dismutase inhibitor. Inclusion of an SOD inhibitor in lucigenin-based chemiluminescence is necessary to have a measurable signal, otherwise superoxide is rapidly destroyed. However, DDC has been reported to inhibit cytochrome P450 (Saadeddin et al., 2004). This will take further investigation.

**Cytochrome P450 Metabolism of 5-HT to NO.** The cytochrome P450 3A inhibitor KTZ did potentiate 5-HT-induced contraction, and this potentiation was specific to 5-HT. However, KTZ did not reveal increases in 5-HT-stimulated O$_2^-$ production. One other possible explanation for our negative findings was the inclusion of DDC as a superoxide dismutase inhibitor. Inclusion of an SOD inhibitor in lucigenin-based chemiluminescence is necessary to have a measurable signal, otherwise superoxide is rapidly destroyed. However, DDC has been reported to inhibit cytochrome P450 (Saadeddin et al., 2004). This will take further investigation.

**TABLE 2**

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>EC$_{50}$ Value</th>
<th>log mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>6.0 ± 0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>KTZ (10 μM)</td>
<td>6.7 ± 0.3*</td>
<td>0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>1-NNA (100 μM)</td>
<td>6.0 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.8 ± 0.2</td>
<td></td>
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<tr>
<td>ABT (0.5 mM)</td>
<td>5.7 ± 0.2</td>
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</tr>
<tr>
<td>Vehicle</td>
<td>5.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Clostrimazole (10 μM)</td>
<td>5.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>5.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>14,15-EEZE (10 μM)</td>
<td>5.6 ± 0.1*</td>
<td></td>
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</tbody>
</table>

* Statistically significant difference from the respective vehicle.
normal arteries. In contrast, inhibition of catalase inhibited, rather than potentiated, 5-HT-induced contraction, suggesting that, in arteries, NO is not produced by cytochrome P450 metabolism of 5-HT, at least not in the manner previously thought (Fradette et al., 2004). Further evidence that KTZ does not potentiate 5-HT-induced contraction by inhibiting cytochrome P450 metabolism of 5-HT to NO is that ABT, a general cytochrome P450 inhibitor, and clotrimazole, an imidazole P450 inhibitor like KTZ, did not have the same effect on 5-HT-induced contraction. Together, these data suggest 5-HT is not metabolized by cytochrome P450 to NO in rat aorta, thus the effects of KTZ on 5-HT-induced contraction are not dependent on this metabolism. We attempted to measure NO production in tissues stimulated with 5-HT using an NO-selective electrochemical probe from WPI (Sarasota, FL), but we were unsuccessful in our attempts.

Mechanism of Action of KTZ. Although l-NNa and KTZ similarly potentiated 5-HT-induced contraction, we demonstrated that the effects of KTZ on 5-HT-induced contraction were not dependent on endothelium and that KTZ did not inhibit NOX present in the endothelium. Isoforms of NOX exist in vascular smooth muscle (Brophy et al., 2000); however, it is unlikely that KTZ acts by inhibiting NOX present in vascular smooth muscle because l-NNa did not affect 5-HT-induced contraction in endothelium-denuded aorta. Thus, the potentiation of 5-HT-induced contraction by KTZ does not seem to be dependent on NO derived from NOX. Furthermore, the effects of KTZ do not depend on NADPH-derived O$_2^\cdot$ since apocynin did not correct the enhanced 5-HT-induced contraction caused by KTZ.

Cytochromes P450 contribute to arterial tone independently of NO production. Cytochrome P450 metabolize arachidonic acid to EETs, which causes relaxation of blood vessels, and they are putative endothelium-derived hyperpolarizing factors (Roman, 2002). Using the EET antagonist 14,15-EEZE, we showed that EETs do not play a significant role in 5-HT-induced contraction of endothelium-denuded aorta. 14,15-EEZE does not alter synthesis or the constrictor effect of 20-HETE (Gauthier et al., 2002), but it does have intrinsic vasodilator properties (Harrington et al., 2004), which could explain the attenuated 5-HT-induced contraction in the presence of 14,15-EEZE.

KTZ and 5-HT$_{2A}$ Receptors. KTZ selectively potentiated 5-HT-induced contraction in a manner that was not dependent on cytochrome P450 inhibition. KTZ also potentiated contraction elicited by the 5-HT$_2$ receptor agonist α-methyl-5-HT, suggesting that the effects of KTZ are dependent on 5-HT$_{2A}$ receptor activation. However, there is a lack of data on the affinity of KTZ for 5-HT receptors (see Psychoactive Drug Screening Program K$\text{a}$ database at http://pdpd.med.unc.edu/pdpd.php), and at the concentrations we used, KTZ did not elicit contraction of these vessels, suggesting KTZ is not an agonist at the 5-HT$_{2A}$ receptor. One possibility is KTZ is an antagonist of the relaxant 5-HT$_1$ receptor, although it has been difficult to verify antagonists of this receptor, and no binding data exist describing the interaction of KTZ and 5-HT receptors. Conversely, KTZ might potentiate 5-HT-induced contraction by activating or up-regulating second messengers in the 5-HT$_{2A}$ receptor signal transduction cascade. Since KTZ specifically potentiated 5-HT-induced contraction, KTZ would have to affect a messenger that is specific to the 5-HT signaling cascade. Because KTZ potentiated 5-HT-induced contraction in a manner that was not dependent on classical actions of KTZ, namely, cytochrome P450 or NOX, KTZ might be acting on a novel pathway of 5-HT-induced contraction. Thus, clarifying the mechanism of action of KTZ will further our understanding of the actions of 5-HT in the cardiovascular system.

In our studies, 5-HT did not induce production of O$_2^\cdot$ Our studies were undertaken in animals with normal blood pressure, but in hypertensive animals, blood vessels become hyperresponsive to 5-HT (McGregor and Smirk, 1970; Turla and Webb, 1989), and the primary 5-HT receptor mediating contraction switches from the 5-HT$_{2A}$ to 5-HT$_{3B}$ (Watts, 1998). Since elevated O$_2^\cdot$ levels are associated with hypertension and O$_2^\cdot$ may mediate the development of hypertension (Touyz and Schiffrin, 2004), it would be worthwhile to investigate whether 5-HT can stimulate O$_2^\cdot$ in these conditions.

In summary, KTZ acts in a novel way to potentiate 5-HT-induced contraction. Contrary to our hypothesis, we found 5-HT does not stimulate production of O$_2^\cdot$ and that it is not metabolized by cytochrome P450 into NO. We have diverged the effects of KTZ from inhibition of cytochrome P450 and NOX. KTZ potentiated α-methyl-5-HT-induced contraction, suggesting KTZ may be interacting directly with 5-HT receptors. Importantly, KTZ does not affect contraction in general, but it specifically potentiates contraction elicited by 5-HT and it may be acting on a novel component of 5-HT-induced contraction.

References


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