Serotonin 5-HT(2B) receptor blockade prevents reactive oxygen species-induced cardiac hypertrophy in mice.

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Abstract—We established previously that 5-HT_{2B} receptors are involved in cardiac hypertrophy through the regulation of hypertrophic cytokines in cardiac fibroblasts. Moreover, the generation of reactive oxygen species and tumor necrosis factor-α through the activation of reduced nicotinamide-adenine dinucleotide phosphate [NAD(P)H] oxidase has been implicated in cardiac hypertrophy. In this study, we investigated whether 5-HT_{2B} receptors could be involved in the development of cardiac hypertrophy associated with superoxide anion production. Therefore, we measured the effects of serotonergic 5-HT_{2B} receptor blockade on left-ventricular superoxide anion generation in 2 established pharmacological models of cardiac hypertrophy, ie, angiotensin II and isoproterenol infusions in mice. Angiotensin II infusion for 14 days increased superoxide anion concentration (+32%), NAD(P)H oxidase maximal activity (+84%), and $p47^{phox}$ NAD(P)H oxidase subunit expression in the left ventricle together with hypertension (+37 mm Hg) and cardiac hypertrophy (+17% for heart weight:body weight). The 5-HT_{2B} receptor blockade by a selective antagonist (SB215505) prevented the increase in cardiac superoxide generation and hypertrophy. Similarly, infusion for 5 days of isoproterenol increased left-ventricular NAD(P)H oxidase activity (+48%) and cardiac hypertrophy (+31%) that were prevented by the 5-HT_{2B} receptor blockade. Finally, in the primary culture of left-ventricular cardiac fibroblasts, angiotensin II and isoproterenol stimulated NAD(P)H oxidase activity. This activation was prevented by SB215505. These findings suggest that the 5-HT_{2B} receptor may represent a new target to reduce cardiac hypertrophy and oxidative stress. Its blockade affects both angiotensin II and β-adrenergic trophic responses without significant hemodynamic alteration. (***Hypertension*. 2008;52:301-307.)**

Key Words: 5-HT_{2B} ■ NAD(P)H oxidase ■ superoxide anion ■ angiotensin ■ adrenergic ■ cardiac ■ hypertrophy

Pathological left ventricular hypertrophy has been associated with increased production of reactive oxygen species (ROS). Recent works have shown that antioxidants inhibit in vitro cardiomyocyte hypertrophy and in vivo aortic banding-induced left ventricular hypertrophy.[1] The cardiac ROS production can be triggered by factors such as angiotensin II (Ang II), catecholamines, endothelin-1, tumor necrosis factor-α (TNF-α), and serotonin but also by mechanical stretch. Among these factors, those that signal through the G_q/PLC pathway seem to play a crucial role in the initiation and maintenance of cardiac hypertrophy and are known to stimulate the cardiac ROS generation through the phagocyte-type reduced nicotinamide-adenine dinucleotide phosphate [NAD(P)H] oxidase.[2] The $gp91^{phox}$-containing NAD(P)H oxidase plays a pivotal role in the response to Ang II via a pathway involving protein kinase C, c-Src, and phosphatidylinositol 3-kinase.[3] NAD(P)H oxidase reduces oxygen O_2, leading to the formation of superoxide anion ($O_2^{\cdot-}$), which can be either dismutated spontaneously to hydrogen peroxide or in a reaction involving superoxide dismutase (SOD).

The 5-HT_{2B} receptor (5-HT_{2B}R) is a G_q/G_{11} protein-coupled receptor that has been shown to be functionally coupled to ROS synthesis through NAD(P)H oxidase stimulation in a neuroectodermal cell line (1C11).[4] Interestingly, 5-HT_{2B}Rs appear to control the TNF-α shedding in the extracellular space via NAD(P)H oxidase–dependent TNF-α–converting enzyme activation. This 5-HT_{2B}R–dependent NAD(P)H oxidase activation could contribute to the previously described effect of 5-HT_{2B}R blockade on TNF-α release by ventricular fibroblasts after isoproterenol (ISO) stimulation. We established that 5-HT_{2B}Rs are essential for ISO-induced cardiac hypertrophy and are involved in the regulation of hypertrophic cytokines, interleukin-6, interleukin-1β, and TNF-α production by cardiac fibroblasts.[5] We hypothesized that

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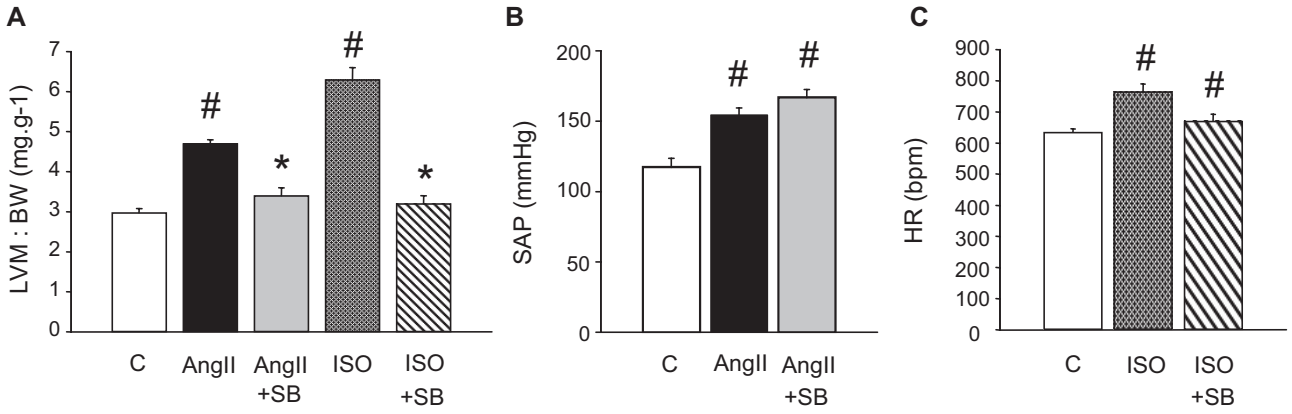

Figure 1. In mice, SB215505 (SB) prevents Ang II- and ISO-induced left-ventricular mass:body weight ratio (LVM:BW) increase (A) without effect on the systolic arterial pressure (SAP) during Ang II infusion (B) and ISO-induced tachycardia (HR; difference: 9%; $P=0.07$; C). #$P<0.05$ vs controls (C: vehicle-infused); *$P<0.05$ vs Ang II (n=8) or ISO alone (n=9; all controls were pooled in A). Statistical analysis was performed on each corresponding control group (n=8 each); $P>0.05$ between the 2 control groups.

5-HT_{2B}R blockade could affect NAD(P)H-oxidase function and, therefore, participate in the modulation of hypertrophic pathways implying this key enzyme.

The aim of the present study was to determine whether 5-HT_{2B}Rs could participate in $O_2^{\cdot-}$ generation during the course of pharmacological-induced cardiac hypertrophy by ISO and Ang II. Its roles in the regulation of the oxidative balance between NAD(P)H oxidase and SOD activities and in the $O_2^{\cdot-}$ production by left-ventricular fibroblasts have been investigated.

Methods

Animals

Studies were performed in 129S1/ScImJ mice in accordance with the Canadian Council for Animal Care Guidelines and monitored by the ethical committee for experimental research from the Clinical Research Institute of Montreal Canada (No. 2004-11).

Induction of Cardiac Hypertrophy by ISO and Ang II

Mice were infused by either vehicle, ISO (30 $mg \cdot kg^{-1} \cdot d^{-1}$), or Ang II (0.2 $mg \cdot kg^{-1} \cdot d^{-1}$; please see the detailed procedures available in the online data supplement at http://hyper.ahajournals.org). The following drugs were tested on cardiovascular responses to ISO and/or Ang II: the 5-HT_{2B}R antagonist SB215505 (1 $mg \cdot kg^{-1} \cdot d^{-1}$), the antagonist of β-adrenergic receptors propranolol (5 $mg \cdot kg^{-1} \cdot d^{-1}$), or the NAD(P)H oxidase inhibitor apocynin (1.5 mmol/L, drinking water).

Heart rate and systolic arterial pressure were recorded by the tail-cuff method (Visitech), and transthoracic echocardiograms were performed in 2% isoflurane anesthetized mice, as described previously.[5] After euthanasia, the heart was weighed and quickly frozen after separation of right and left ventricles and atria. In some experiments, the abdominal aorta was sampled.

$O_2^{\cdot-}$ Measurements

Basal and NAD(P)H-stimulated $O_2^{\cdot-}$ ventricular productions were measured twice for each ventricle using the lucigenin-enhanced chemoluminescence method as described previously[6] and related to milligrams of tissue. For Ang II–treated mice, the aortic O_2^- production was also measured. Approximately 2 to 3 mg of tissue sample were placed in a glass vial containing 2 mL of a lucigenin solution (5 μmol/L). After the measurement of basal luminescence, 10^{-4} mol/L of NAD(P)H were added to the same vial to evaluate the maximal O_2^- production by NAD(P)H oxidase.

SOD Activity Measurements

SOD enzymatic activity was measured according to the hematoxylin method of Chattopadhyay et al.[7] The enzymatic reaction was assessed with 10 μg of proteins and 50 μmol/L of hematoxylin with a UV-visible recording spectrophotometer (Shimadzu Corp).

gp47-phox Ventricular Expressions

Ventricular tissue was crushed in liquid nitrogen. Lysis and Western blots were performed as described previously[6] with 35 μg of proteins loaded on gels (antibodies from Santa Cruz Biotechnology, gp47-phox rabbit [H-195] sc-14015).

Adult Cardiac Fibroblasts Primary Culture

Ten- to 12-week-old mouse left ventricle fibroblasts were cultured as described previously[5] and transferred to serum-free medium before a 24-hour pharmacological stimulation. Cells were treated either with serum-free culture medium only (controls) or added to Ang II (10^{-7} mol/L) or ISO (10^{-5} mol/L) alone or simultaneously with the 5-HT_{2B}R antagonist, SB215505 (10^{-7} mol/L). After treatments, cells were washed in oxygenated Krebs-Hepes buffer. The $O_2^{\cdot-}$ production was measured with the lucigenin method and adjusted by the protein concentration (counts per minute per microgram) of the samples.

Data Analysis and Statistics

Data are expressed as means±SEMs. Statistical comparisons were made by ANOVA followed by the Bonferroni's method with the GraphPad Prism program (GraphPad Software). A value of $P<0.05$ was considered significant.

Results

Effect of the 5-HT_{2B}R Antagonist, SB215505, on Chronic Ang II–Induced Cardiovascular Alterations

The Ang II infusion induced cardiac hypertrophy as assessed by echocardiographic measurement of the left ventricle mass:body weight ratio (+52% over controls; Figure 1A) and direct measurement of the heart weight:body weight ratio (+17% versus controls; Table). A simultaneous 37-mm Hg increase in blood pressure (Figure 1B) and no significant heart rate change (595±14 bpm in Ang II versus 605±22 bpm in controls; $P>0.05$) were observed. In response to the afterload increase, the fractional shortening was also increased (Table). SB215505 reduced the Ang II–induced left ventricular hypertrophy (Table and Figure 1A) without affecting the increased blood pressure that remained elevated

Table. Echocardiographic Parameters, Body Weight, and Cardiac Mass

Groups	BW, g	CO, mL · min^{-1}	EDD, mm	ESD, mm	FS, %	PWd, mm	Sd, mm	HW, mg	HW/BW, mg · g^{-1}
C Ang II (n=8)	24±1	27±2	3.65±0.07	2.28±0.08	37±2	0.57±0.02	0.69±0.02	130±3	5.4±0.1
Ang II (n=8)	22±1	26±2	3.68±0.08	2.04±0.07†	44±2†	0.70±0.02†	0.88±0.02†	136±4	6.3±0.1†
AGSB (n=8)	23±1	24±1	3.56±0.11	1.89±0.12†	47±2†	0.62±0.04*	0.77±0.02*†	137±3	5.9±0.2*†
C ISO (n=8)	23±1	25±1	3.61±0.06	2.19±0.08	39±2	0.53±0.01	0.63±0.01	124±4	5.5±0.1
ISO (n=9)	24±1	26±2	4.19±0.07†	2.91±0.13†	33±3	0.88±0.03†	0.94±0.02†	174±5†	7.2±0.2†
ISOPro (n=6)	23±1	21±2*	3.67±0.2*	2.42±0.21*	34±3	0.56±0.02*	0.72±0.02*	142±3*†	6.1±0.1*†
ISOApo (n=8)	24±1	31±2*	3.76±0.08*	2.41±0.14*	37±3	0.61±0.01*	0.72±0.01*	152±4*†	6.4±0.1*†
ISOSB (n=8)	24±1	32±2*	3.46±0.11*	2.15±0.1*	38±2	0.66±0.02*	0.75±0.03*	164±5†	6.8±0.2*†

C indicates control; SB, SB215505; Pro, propranolol; Apo, apocynin; BW, body weight; CO, cardiac output; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; PWd, posterior wall thickness in diastole; Sd, septum wall thickness in diastole; HW, heart weight; HW/BW, heart weight:body weight ratio.

*$P<0.05$ vs Ang II or ISO alone.

†$P<0.05$ vs controls (C).

by 40 mm Hg over controls (Figure 1B). The heart rate was unaffected by the SB215505 treatment (584±17 bpm; $P>0.05$ versus controls), and the fractional shortening was still increased compared with controls (Table).

Effect of the 5-HT$_{2B}$R Antagonist, SB215505, on Chronic ISO-Induced Cardiovascular Alterations

ISO induced a cardiac hypertrophy measured by echocardiography (+117% over controls for left ventricle mass:body weight ratio; Figure 1A) and direct measurement of the heart weight:body weight ratio (+31%; Table). This hypertrophy was associated with left ventricular dilatation, as shown by the increase in end-diastolic diameter (+16%; Table and Figure 2) and tachycardia (+17%; $P<0.05$; Figures 1C and 2). No significant changes in blood pressure (115±7 mm Hg in controls versus 114±6 mm Hg in ISO; $P>0.05$) and cardiac contractility (fractional shortening) were detected. The cardiac output was preserved (Table). To confirm that these effects were mediated by β-adrenergic receptors, mice were simultaneously treated by ISO and the nonselective β-adrenergic antagonist propranolol. This compound reduced cardiac hypertrophy (Table) and tachycardia induced by ISO (744±38 bpm in ISO versus 619±24 bpm in ISO+propranolol; $P<0.05$) but also slightly reduced the cardiac output. SB215505 prevented the left ventricular hypertrophy caused by ISO (Figure 1A and Table) and the cardiac dilatation, the end-diastolic diameter (end-diastolic diameter) being similar to controls (Table and Figure 2). This prevention of cardiac hypertrophy was obtained without cardiodepression (Table) or effect on the blood pressure (119±6 mm Hg in ISO+SB215505 versus 115±7 mm Hg in controls; $P>0.05$). We also demonstrated that the cardiac alterations induced by ISO were dependent on NAD(P)H oxidase activation, because the NAD(P)H oxidase inhibitor, apocynin, prevented ISO-induced hypertrophy (Table). Experiments on Nox2$^{-/-}$ mice showed that the NAD(P)H oxidases involved did not include the Nox2 subunit (please see the data supplement).

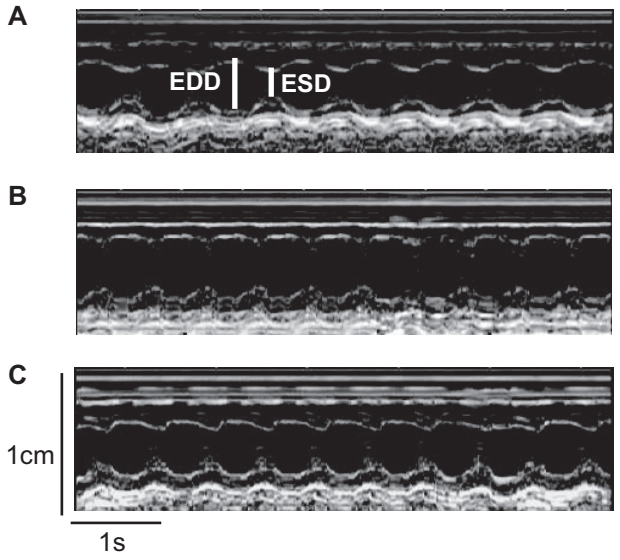

Figure 2. Short-axis view of the left ventricle (M-mode) in control (A), ISO-infused (B), and ISO+SB215505–infused (C) mice showing a prevention of cardiac hypertrophy and ventricular dilatation by SB215505. EDD indicates end-diastolic diameter; ESD, end-systolic diameter.

Involvement of the 5-HT$_{2B}$R and NAD(P)H Oxidases on Cardiac $O_2^{\cdot-}$ Production in Ang II/ISO-Induced Cardiovascular Alterations

Ang II increased the basal $O_2^{\cdot-}$ generation, as well as the NADPH-stimulated NAD(P)H oxidase activity in the left ventricle (Figure 3A and 3B). SB215505 completely normalized $O_2^{\cdot-}$ concentrations in the left ventricle. Ang II induced an increase of the p47^{phox} subunit expression, which was not affected by the simultaneous SB215505 treatment (Figure 4). In the aorta, Ang II also increased basal (623±54 cpm · mg^{-1} in controls to 1145±88 cpm · mg^{-1} in Ang II; $P<0.05$) and NAD(P)H oxidase-mediated (11 8076±23 709 cpm · mg^{-1} in controls to 146 749±10 559 cpm · mg^{-1}; $P<0.05$) $O_2^{\cdot-}$ concentration, but SB215505 was without effect on this Ang II response (820±107 cpm · mg^{-1} in basal and 181 449±19 596 cpm · mg^{-1} after NAD(P)H; $P>0.05$ versus Ang II alone). To verify that the SB215505 effects were not related to a nonspecific antioxidant property, we showed in an in vitro based assay that, in contrast to glutathione and ascorbic acid, it was unable to reduce the level of spontaneous hematoxylin oxidation (please see the data supplement). Moreover, mice subcutaneously infused with 1 mg.kg^{-1} SB215505 alone (5 or 14 days), did not demonstrate any decrease in basal or

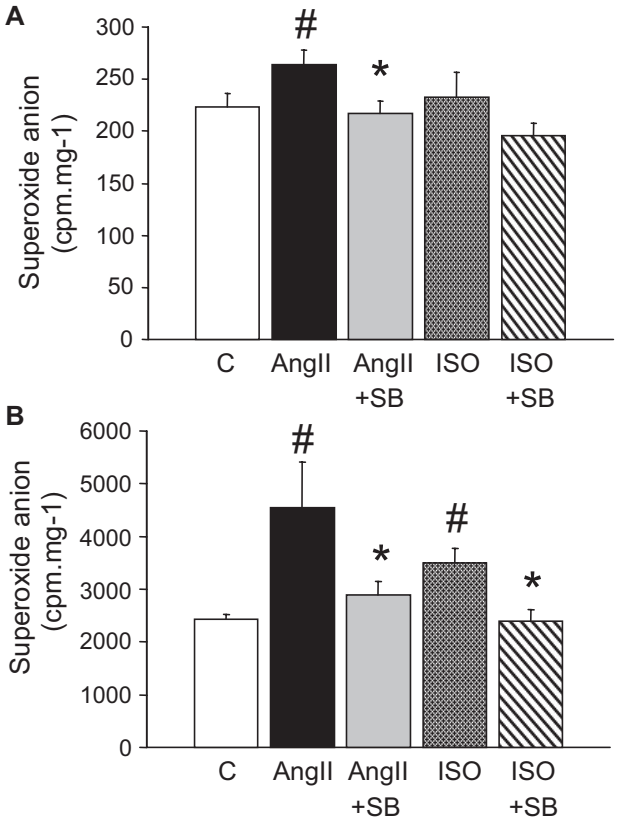

Figure 3. Effects of pharmacological blockade (SB215505; SB) of the 5-HT_{2B}R on left ventricular $O_2^{\cdot-}$ concentration in basal (A) and NAD(P)H-stimulated (B) conditions after Ang II and ISO infusions. #$P<0.05$ vs controls (C: vehicle-infused); *$P<0.05$ vs Ang II or ISO alone, respectively (all controls were pooled). Statistical analysis was performed on each corresponding control group (n=8 each); $P>0.05$ between the 2 control groups.

NAD(P)H-stimulated $O_2^{\cdot-}$ ventricular concentration (please see the data supplement). In conclusion, the pharmacological blockade of 5-HT_{2B}Rs abolished the NAD(P)H oxidase overactivation and, therefore, the $O_2^{\cdot-}$ generation induced by Ang II in the left ventricle.

In contrast to Ang II, the chronic ISO infusion did not modify the basal concentration of $O_2^{\cdot-}$ in the left ventricle (Figure 3A). However, when the maximal activity of NAD(P)H oxidases was tested by the addition of a saturating NAD(P)H concentration (100 μmol/L), we observed a 48% increase in the left ventricular $O_2^{\cdot-}$ production as compared with control animals (Figure 3B). SB215505 prevented the ISO-induced NAD(P)H oxidase activation without significant change in the basal $O_2^{\cdot-}$ concentration after ISO (Figure 3). To analyze whether the increase in left-ventricular NAD(P)H oxidase activity was because of a change in NAD(P)H complex expression, the $p47^{phox}$ subunit expression was assessed by Western blots. ISO induced a 20% ($P<0.05$) increase in left ventricular $p47^{phox}$ subunit expression (Figure 4). Similar to Ang II+SB215505-treated mice, this overexpression was not affected by SB215505 (Figure 4). Taken together, these data show that Ang II and ISO induced an increase in left ventricular $O_2^{\cdot-}$ generation in association with an increase in the $p47^{phox}$ NAD(P)H oxidase subunit expression.

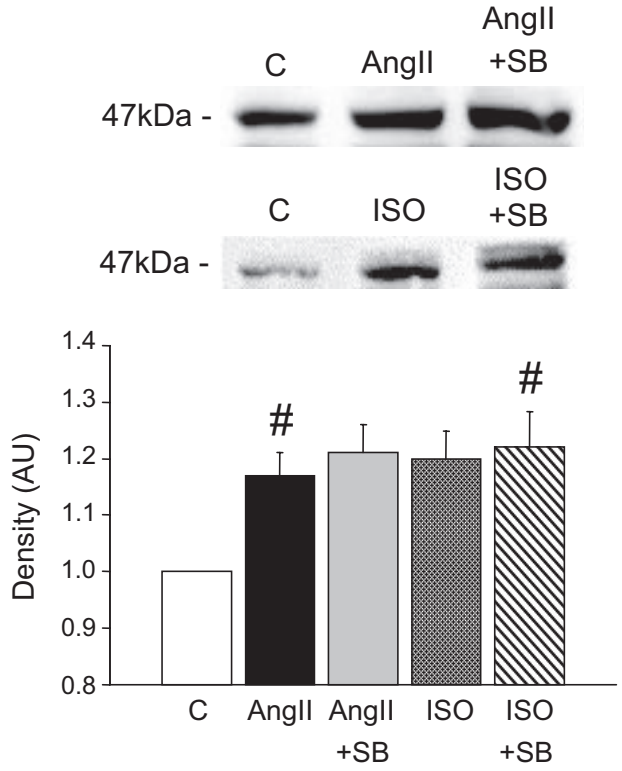

Figure 4. Overexpression of the $p47^{phox}$/NAD(P)H oxidase subunit in mice after Ang II and ISO that is not affected by SB215505 (SB). #$P<0.05$ vs controls (C: vehicle infused; n=6).

Effect of SB215505 on SOD Activity After Ang II and ISO Stimulations

To explain the difference between ISO and Ang II in basal $O_2^{\cdot-}$, we postulated a different counterregulatory mechanism by SOD: the enzyme that triggers the dismutation of $O_2^{\cdot-}$ to oxygen peroxide and is therefore involved in the $O_2^{\cdot-}$ clearance. ISO induced a 54% increase in the activity of SOD (Figure 5) that was prevented by the simultaneous administration of SB215505, indicating that the reducing effect of this drug on left ventricular $O_2^{\cdot-}$ concentration is rather attributable to a regulation of NAD(P)H oxidase activity than to an increased SOD activity. In contrast to ISO, the SOD activity was not significantly modified by Ang II. Therefore, the increased NAD(P)H oxidase–mediated $O_2^{\cdot-}$ concentration by Ang II was not limited by a simultaneous augmentation of SOD activity, as observed during ISO stimulation. Similarly, when SB215505 was simultaneously administered with Ang II, the SOD activity was not affected.

Effect of 5-HT_{2B}R Blockade on $O_2^{\cdot-}$ Production and NAD(P)H Oxidase Activity in Primary Culture of Left Ventricular Fibroblasts

We analyzed the role of the 5-HT_{2B}R on NAD(P)H oxidase activity after a 24-hour stimulation with either Ang II or ISO in left-ventricular fibroblasts. Ang II and ISO did not affect the basal $O_2^{\cdot-}$ production (data not shown) but induced, respectively, a 34% and a 42% increase in NAD(P)H oxidase–mediated $O_2^{\cdot-}$ concentration compared with controls (Figure 6). These increases were completely prevented by the simultaneous SB215505 treatment, indicating that 5-HT_{2B}Rs

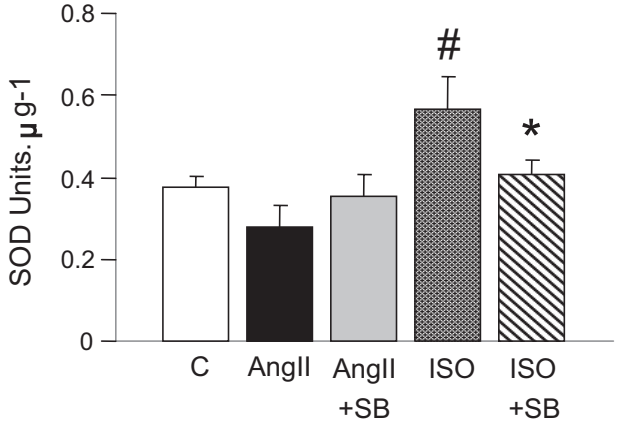

Figure 5. In mice, Ang II and ISO induce a different pattern of regulation of the SOD activity: overactivation of SOD by ISO is prevented by SB when Ang II does not significantly modify this activity. #$P<0.05$ vs controls (C: vehicle-infused; n=13); *$P<0.05$ vs Ang II or ISO alone (n=6).

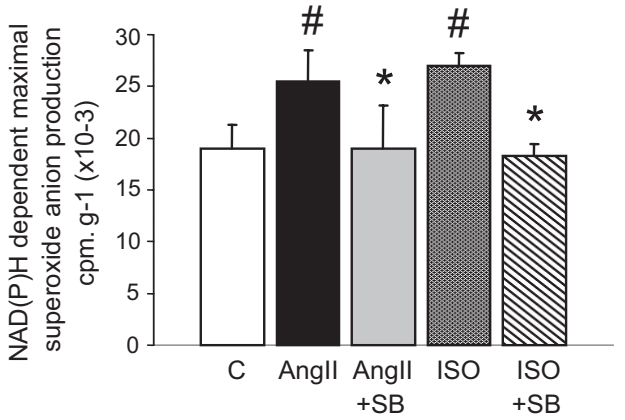

Figure 6. In cultured mouse left ventricular fibroblasts, the pharmacological blockade (SB215505 [10^{-7} mol/L]; SB) of the 5-HT$_{2B}$R prevents the NAD(P)H oxidase–mediated increase in $O_2^{\cdot-}$ production induced by a 24-hour stimulation with Ang II (10^{-7} mol/L) and ISO (10^{-5} mol/L; n=4 in each group). #$P<0.05$ vs controls (n=8); *$P<0.05$ vs Ang II or ISO alone (n=4).

can regulate the NAD(P)H oxidase activity induced by these agonists in cardiac fibroblasts (Figure 6).

Discussion

5-HT$_{2B}$R Blockade Prevents $O_2^{\cdot-}$-Mediated Ang II–Induced Cardiac Hypertrophy

Ang II is recognized as a cardiac hypertrophic factor in vitro, as well as in vivo,[8] and gp91^{phox}/Nox2-mediated ROS production in cardiomyocytes contributes to this response.[9] We tested whether the blockade of 5-HT$_{2B}$Rs could reduce Ang II-induced hypertrophy and cardiac ROS generation in mice. Ang II induced a left ventricular hypertrophy associated with an increase in $O_2^{\cdot-}$ production but no increase of SOD-mediated protection. The blockade of 5-HT$_{2B}$Rs by SB215505 completely prevented the increased left ventricular $O_2^{\cdot-}$ generation in basal and stimulated conditions. The reduction of the NAD(P)H oxidase activity by 5-HT$_{2B}$R blockade does not take place at the expression level, because p47^{phox}-subunit was still overexpressed. This drug was selected because of its higher selectivity (×10) for 5-HT$_{2B}$Rs compared with 5-HT$_{2C}$.[10] Moreover, this drug is a potent antagonist, and we have shown previously[5] that only 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors are expressed in the heart. Interestingly, this antihypertrophic action was achieved without reduction of arterial blood pressure, attesting to the load-independent cardiac antihypertrophic effect of SB215505. In the aorta, Ang II also increased basal and NAD(P)H oxidase–mediated $O_2^{\cdot-}$ concentration, which was insensitive to SB215505, supporting the notion that Ang II type 1 and 5-HT$_{2B}$Rs are not interacting in aorta. Also, SB215505 neither affected the basal $O_2^{\cdot-}$ concentration nor the NAD(P)H oxidase maximal activity in control animals (please see the data supplement), and experiments using hematoxylin autooxidation did not reveal any direct chemical antioxidative properties of the compound. Therefore, SB215505 is not a nonspecific antioxidant and does not directly inhibit the NAD(P)H oxidase complex. These data demonstrate that the 5-HT$_{2B}$R is a key regulator of Ang II–mediated cardiac hypertrophy and NAD(P)H oxidase activity.

Concerns were raised regarding the capacity of lucigenin to self-generate $O_2^{\cdot-}$ through redox cycling. This problem was addressed by using a low concentration of lucigenin (5 µmol/L) as reported by Skatchkov et al[11] and confirmed by Spin-trapping studies. No variation in luminescence was detected when comparing a blank vial filled with a lucigenin solution with or without 100 µmol/L of NAD(P)H.

5-HT$_{2B}$R Blockade Prevents $O_2^{\cdot-}$-Mediated ISO-Induced Cardiac Hypertrophy

We show that the subchronic β-adrenergic activation induces a geometric remodeling of the left ventricle as attested by dilatation. This alteration is completely prevented by the 5-HT$_{2B}$R antagonist without major hemodynamic adverse effects, ie, no reduction of blood pressure or cardiac contractility. In the present work, a slight reduction of the ISO-induced tachycardia (−9.8%) was observed. This reduction was probably not a consequence of a pharmacological effect of this compound on the sinus node, because this drug never reduced the heart rate in any other group but could rather be associated with the prevention of cardiac remodeling.

The role of ventricular oxidative stress after β-adrenoceptor stimulation is still a matter of debate. A recent study has shown that the initial steps of the cardiac hypertrophy after ISO infusion involve $O_2^{\cdot-}$ production,[12] but the cellular origin of $O_2^{\cdot-}$ is unclear. An in vitro study suggested that the stimulation of β-adrenergic receptors located on cardiomyocytes induces hypertrophy, which is not mediated by increased ROS production.[13] In the present work, the NAD(P)H oxidase selective inhibitor, apocynin, prevented the ISO-induced cardiac hypertrophy, suggesting that NAD(P)H oxidase–dependent oxidative stress is involved in this in vivo model. β-Adrenergic receptor stimulation is likely involved, because the nonselective β-blocker propranolol had a similar effect to apocynin. Moreover, we observed an increase of NAD(P)H oxidase maximal activity after ISO infusion. Rathore et al[14] have shown that the increased cardiac oxidative stress induced by ISO was counteracted by a simulta-

neous increase in SOD activity in rats. We reproduced the same result in mice. The physiological relevance of this activation is not yet understood but, 2 aspects could be considered: a deviation of $O_2^{\cdot-}$ to hydrogen peroxide, the last being involved in hypertrophy, or a compensatory mechanism to $O_2^{\cdot-}$ increase that would not be sufficient to prevent cardiac hypertrophy. These questions were addressed recently in the study by Cabassi et al[15] performed on prehypertensive spontaneously hypertensive rats. These animals exhibit an overactivity of the sympathetic nervous system together with increased oxidative stress status and cardiomyocyte hypertrophy. In this model, the SOD mimetic, hydroxytetramethyl piperidinoxyl, was unable to prevent cardiac hypertrophy, indicating that "pushing" $O_2^{\cdot-}$ to hydrogen peroxide neither prevented nor amplified the hypertrophic phenotype. Therefore, in our experimental conditions, the SOD overactivation is probably not sufficient to suppress enough $O_2^{\cdot-}$ production and prevent ventricular hypertrophy. After treatment with a 5-HT_{2B}R antagonist, the SOD activity returned to control values, showing that the reduction of $O_2^{\cdot-}$ concentrations was not because of an increased rate of degradation but rather because of a reduction in the production of $O_2^{\cdot-}$.

The 5-HT_{2B}R Regulates NAD(P)H Oxidase Activation in Cardiac Fibroblasts

In mice knockout for the Nox2 subtype of the $gp91^{phox}$ catalytic subunit of the NAD(P)H oxidase complex ($Nox2^{-/-}$), ISO induced cardiac hypertrophic responses that were prevented by a treatment with the NAD(P)H oxidase inhibitor apocynin (please see http://hyper.ahajournals.org). In cardiac myocytes, the $gp91^{phox}$/Nox2 is the prominent isoform, whereas Nox1 and Nox4 are expressed at lower levels.[12] In $Nox2^{-/-}$ mice, stimulation with ISO induces Nox1 overexpression (please see the data supplement). Other Nox isoforms are also known to be expressed in human cardiac fibroblasts, whereas Nox2 is barely detectable.[16] Therefore, considering the following: (1) apocynin prevents cardiac hypertrophy induced by ISO infusion in $Nox2^{-/-}$ mice; (2) non-Nox2/$gp91^{phox}$ homologues are expressed by fibroblasts; (3) the suppression of Nox2, mainly expressed in cardiomyocytes, cannot suppress ISO-mediated cardiac hypertrophy; and (4) the stimulation of β-adrenergic receptors located on cardiomyocytes induces a ROS-independent hypertrophy,[17] it is likely that noncardiomyocytes play an important role in the cardiac hypertrophic responses to β-adrenergic activation.

Our previous study[5] indicated that fibroblasts constitute a target of hypertrophic responses to β-adrenergic activation, because 5-HT_{2B}R blockers diminished the release of hypertrophic cytokines. We tested the effect of 5-HT_{2B}R blockade in $O_2^{\cdot-}$ production by cardiac fibroblasts during 24-hour stimulations with Ang II or ISO. Ang II and ISO increased the maximal NAD(P)H oxidase–mediated $O_2^{\cdot-}$ production, and this effect was prevented by SB215505. This result indicates a role of this receptor in ISO and Ang II-induced NAD(P)H oxidase activation in these cells. This effect was obtained without stimulation of the receptor by its natural agonist, because in our experimental conditions the serotonin concentration in cell culture medium was found <1 nM at the end of the experiments. Therefore, SB215505, through its interaction with the 5-HT_{2B}R, seems to interfere with a crosstalk among β-adrenergic, 5-HT_{2B}, and Ang II receptors.

Perspectives

The involvement of 5-HT_{2B}Rs in the development of cardiac hypertrophy is a new finding with potential value for the treatment or prevention of the disease. In the present study, the blockade of this receptor can prevent the increase of NAD(P)H oxidase activity and the development of cardiac hypertrophy induced by Ang II type 1 or β-adrenergic receptors, but several questions remain to be answered. Little is known about the basal activity of the 5-HT_{2B}R and the possible agonists involved in its stimulation (serotonin) or regulation in the context of the development of cardiac hypertrophy. Also, the signaling factors responsible for the interactions among Ang II type 1, β-adrenergic, and 5-HT_{2B}Rs must still be identified. A major impact of this work was to demonstrate for the first time that the Gq-coupled 5-HT_{2B}R blockade can modify both Ang II type 1 and β-adrenergic oxidant and hypertrophic responses. The mechanism that triggers this cross-regulation will have to be investigated.

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Disclosures

None.

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