

Role of serotonin 5-HT_{2A} receptors in the development of cardiac hypertrophy in response to aortic constriction in mice

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Abstract Serotonin, in addition to its fundamental role as a neurotransmitter, plays a critical role in the cardiovascular system, where it is thought to be involved in the development of cardiac hypertrophy and failure. Indeed, we recently found that mice with deletion of monoamine oxidase A had enhanced levels of blood and cardiac 5-HT, which contributed to exacerbation of hypertrophy in a model of experimental pressure overload. 5-HT_{2A} receptors are expressed in the heart and mediate a hypertrophic response to 5-HT in cardiac cells. However, their role in cardiac remodeling in vivo and the signaling pathways associated are not well understood. In the present study, we evaluated the effect of a selective 5-HT_{2A} receptor antagonist, M100907, on the development of cardiac hypertrophy induced by transverse aortic constriction (TAC). Cardiac 5-HT_{2A} receptor expression was transiently increased after TAC, and was recapitulated in cardiomyocytes, as observed with 5-HT_{2A} in situ labeling by immunohistochemistry. Selective blockade of 5-HT_{2A} receptors prevented the development of cardiac hypertrophy, as measured by echocardiography, cardiomyocyte area and heart weight-to-

body weight ratio. Interestingly, activation of calmodulin kinase (CamKII), which is a core mechanism in cardiac hypertrophy, was reduced in cardiac samples from M100907-treated TAC mice compared to vehicle-treated mice. In addition, phosphorylation of histone deacetylase 4 (HDAC4), a downstream partner of CamKII was significantly diminished in M100907-treated TAC mice. Thus, our results show that selective blockade of 5-HT_{2A} receptors has beneficial effect in the development of cardiac hypertrophy through inhibition of the CamKII/HDAC4 pathway.

Keywords Serotonin · 5-HT_{2A} receptor · M100907 · Heart · Hypertrophy

Introduction

Serotonin (5-HT), in addition to its fundamental role as a neurotransmitter, is a vasoactive substance that elicits several effects on the cardiovascular system. The myriad of biological actions of 5-HT are mediated by no fewer than 15 receptors divided into seven subtypes (5-HT₁ to 5-HT₇). Among those, 5-HT₂ receptors, which are coupled to the G_{q/11}/PLC signaling pathway, are known to be critical both in the central nervous and cardiovascular systems. 5-HT₂ receptors have generated strong therapeutic interest in a diverse array of pathophysiological implications such as sleep, hallucinogenesis, schizophrenia, appetite control, neuroendocrine secretions, hypertension, migraine and depression.

In the heart, accumulating evidences point toward 5-HT₂ receptors as potential candidates in the development of cardiac hypertrophy. Cardiac hypertrophy is an adaptive response to pressure overload, initiated by hypertension or valvular diseases. When prolonged, cardiac hypertrophy can lead to heart failure, a condition where the diseased

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heart is no longer able to meet the circulatory requirements of the body. 5-HT levels have been demonstrated to be increased in blood from heart failure patients and to be associated with disease severity, which support a role for this neurohormone in cardiac failure (Nigmatullina et al. 2009).

Recently, the consequences of monoamine oxidase A (MAO-A) deletion in blood and cardiac serotonin as well as in cardiac remodeling were evaluated (Lairez et al. 2009). MAO-A constitutes the major metabolic pathway for 5-HT and during substrate degradation, hydrogen peroxide (H₂O₂) is generated, acting as a signaling intermediate in cell hypertrophy and/or death (Mialet-Perez et al. 2007). We hypothesized that MAO-A would play an important role during cardiac hypertrophy, either by regulating 5-HT levels or by its generation of H₂O₂ in the heart. In our study, we found that MAO-A knock-out mice were constantly exposed to elevated levels of blood and cardiac 5-HT, and demonstrated exacerbation of cardiac hypertrophy in response to pressure overload. We identified the 5-HT_{2A} receptor as a major component involved in the exacerbation of hypertrophy in response to elevated levels of 5-HT (Lairez et al. 2009). In vitro, additional evidences demonstrated that 5-HT_{2A} receptors were expressed in cardiac cells and triggered a hypertrophic response to 5-HT, through the involvement of transient receptor potential canonical 1 (TRPC1) channels and calcineurin/NFAT activation (Bush et al. 2004; Villeneuve et al. 2009; Vindis et al. 2010). In addition, further studies demonstrated that during cardiac remodeling, increased expression of 5-HT_{2A} receptors generated a functional inotropic response to 5-HT (Qvigstad et al. 2005).

At present, the consequences of selective inhibition of 5-HT_{2A} receptors in vivo on the development of cardiomyocyte hypertrophy and the signaling pathways associated are not well defined. To address this question, we used M100907, an antagonist with high potency and selectivity toward 5-HT_{2A} receptors, and measured its effect on the development of cardiac hypertrophy in a model of pressure overload induced by transverse aortic constriction (TAC) in mice. We found that selective inhibition of 5-HT_{2A} receptors with M100907 prevented the development of cardiac hypertrophy following pressure overload and inhibited the activation of the calmodulin-dependent kinase II (CaMKII)-histone deacetylase 4 (HDAC4) hypertrophic pathway.

Methods

Aortic banding

C57Bl6J mice (Janvier, France) were housed in a pathogen-free facility and handled in accordance with the

procedures outlined in Council Directive 86/609/EEC. 8-week-old male mice were anesthetized with isoflurane (4 %), ventilated, and the left thorax was opened through a small thoracic window. Aortic constriction (TAC) was performed by ligating the transverse aorta under a dissecting microscope. For this purpose, two knots were made on the thread which was carefully introduced around the transverse aorta. Both knots were tied together to perform aortic constriction (27-gauge needle circumference). Age-matched animals underwent identical surgical procedure except for ligation of the aorta (sham-operated mice).

Experimental protocol

Animals were randomly categorized into four groups: (a) sham vehicle ($n = 5$), (b) TAC vehicle ($n = 10$), (c) sham M100907 ($n = 5$), and (d) TAC M100907 ($n = 10$). Mice were treated for 3 months with the 5-HT_{2A} receptor antagonist M100907 ((R)-(+)-a-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol) (0.1 mg/kg/day, dissolved in a minimum volume of 0.01 N hydrochloric acid, further diluted to its final concentration using 0.9 % saline, and adjusted to pH 7.5) or vehicle, administered with 0.2-ml Alzet mini-osmotic pumps (Charles River labs, L'Arbresle, France) placed intraperitoneally. Mini-osmotic pumps were replaced every month. Animals were sacrificed 1 or 3 months after TAC.

Echocardiography

Animals were anesthetized with 2 % isoflurane and examined with non-invasive echocardiography (echocardiograph Vivid 7 ultrasound, GE). Cardiac ventricular dimensions were measured on M-mode images at least five times for the number of animals indicated.

Invasive hemodynamic study

Animal anesthesia was induced by intraperitoneal injection of ketamine (125 mg/kg) and xylazine (5 mg/kg) and maintained with 1 % isoflurane in oxygen flow (0.1 l/min). Mice were orally intubated with a 20-G catheter and ventilated using a Minivent 845 respirator (Harvard Apparatus, Les Ulis, France); the tidal volume and respiration rate were, respectively, 200 μ l and 150 bpm. Left ventricular apex was reached through the left 5th intercostal space and punctured with a 26-G needle, following the septal axis. A high-fidelity Millar Mikro-Tip pressure catheter (SPR-671, ADInstruments Ltd., Oxford, UK) was then inserted into this pre-hole and left ventricular pressure was recorded for 10 min using a Bridge Amp (ML221) connected to a PowerLab 8/30 acquisition system (ML870, ADInstruments). Data were analyzed with LabChart Pro v7.3.3

software (ADInstruments), using five consecutive 30 s periods.

Western blot

Ventricular homogenates were lysed in RIPA buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1 % Triton X-100, 1 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 5 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin, 1 µg/ml aprotinin). Protein extracts were resolved by SDS-polyacrylamide gel electrophoresis, transferred onto PVDF membranes (Millipore, Molsheim, France). Then, membranes were probed with the indicated primary antibodies and revealed with the secondary antibodies coupled to horseradish peroxidase using the ECL chemoluminescence kit. Membranes were then stripped and re-probed with anti-GAPDH antibody to control equal loading of proteins. Primary antibodies for phospho-Ser632-HDAC4, HDAC4 and CamKII were from Santa-Cruz Biotechnology (Heidelberg, Germany). Primary antibody for 5-HT_{2A} receptor was from GenScript (Paris, France). Primary antibody for phospho-Thr286-CamKII was from Cell Signaling (Saint-Quentin, France).

Histological analysis

Ventricles were incubated in Carnoy's fixative solution (ethanol 60 %, chloroform 30 %, acetic acid 10 %), embedded in paraffin and transversally sectioned. 5 µm tissue sections were stained with H&E. Cardiomyocyte diameter was evaluated after coloration with H&E (250–300 cells counted per heart) on left ventricle.

Immunohistochemistry

Paraffin-embedded tissue sections were first de-waxed in toluene and rehydrated through a series of graded ethanol washes before endogenous peroxidase blockage. Then, they were incubated in blocking buffer (1 % BSA in TBS-T) for 10 min. Specific primary antibodies were incubated (1 h at room temperature) on mouse sections for the detection of 5-HT_{2A} receptor (Genscript, 1:50). For visualization, we used (DAB) chromogen detection system with secondary HRP anti-rabbit antibody. Negative controls for the immunohistochemical procedures included substitution of the primary antibody with non-immune sera.

Real-time RT-PCR

Extraction of RNA was performed using column affinity purification (Macherey-Nagel, Hoerd, France). First-strand cDNA was synthesized using the superscript II RT-PCR

system (Invitrogen, Cergy Pontoise, France) with random hexamers. Negative controls without reverse transcriptase were made to verify the absence of genomic DNA contamination. Real-time PCR was performed on a StepOne-Plus system (Applied Biosystem, Courtaboeuf, France) in 96-well plates. A 1/10 dilution of cDNA (5 µl) from RT reaction was mixed with specific primers and SYBR green mix (Eurogentec, Angers, France). ANP, *NppaF*: 5'-AGAGTGGGCAGAGACAGCAAA-3', *NppaR*: 5'-AAGGCCAAGACGAGGAAGAAG-3'; β-MHC, *Myh7F*: 5'-AGGTGGCTCCGAGAAAGGAA-3', *Myh7R*: 5'-TGAGCCTTGGATTCTCAAACGT-3'; αSK-actin, *Acta1F*: 5'-TACCACCGGCATCGTGTTG-3', *Acta1R*: 5'-CCAGGTCCA-GACGCATGAT-3'; Collagen III, *Coll 3F*: 5'-ACGTAGATGAATTGGGATGCAG-3', *Coll 3R*: 5'-GGGTTGGGCAGTCTAGTG-3'.

Statistical analysis

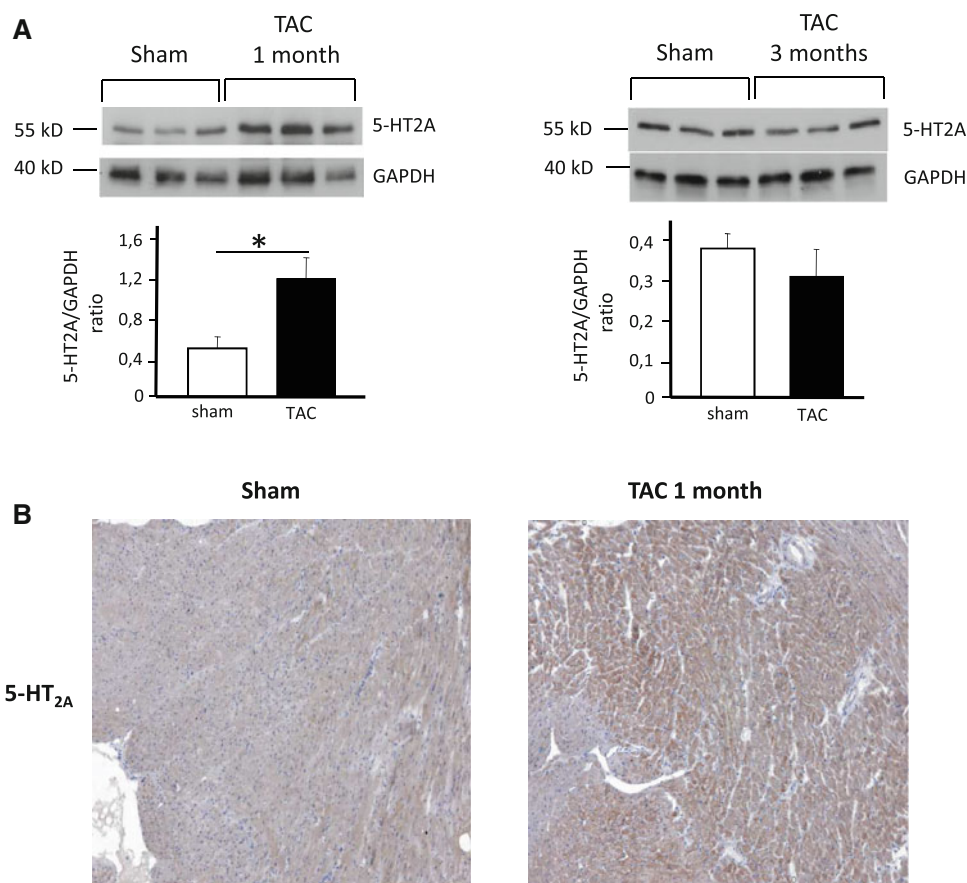
Results are expressed as mean ± SEM. Experimental groups were compared using Student's *t* test, 1-way ANOVA or 2-way ANOVA followed by Bonferroni's post hoc test, as appropriate. A value of *p* < 0.05 was considered significant.

Results

Transient upregulation of 5-HT_{2A} receptor during the development of cardiac hypertrophy

To better understand the relevance of 5-HT_{2A} receptor in cardiac hypertrophy, we followed the time course of regulation of this receptor by Western blot in the model of pressure overload induced by TAC in mice. Pressure overload causes progressive left ventricle hypertrophy, which can lead to subsequent cardiac dilatation and failure in the later stage. As shown in Fig. 1a, 5-HT_{2A} receptor expression was significantly increased in ventricles of TAC-operated mice at 1 month, compared to sham-operated hearts. However, in the later stage of hypertrophy, 3 months after TAC, 5-HT_{2A} receptor expression was slightly decreased (non-significant) compared to sham mice. Next, we investigated the localization of cardiac 5-HT_{2A} receptors by immunohistochemistry in ventricles from sham or TAC mice at 1 month. A good labeling of 5-HT_{2A} receptors was observed in ventricular myocytes of sham-operated mice, which was compatible with binding values measured in previous studies (Fig. 1b) (Jaffre et al. 2009). During cardiac hypertrophy, 5-HT_{2A} receptor staining became more pronounced in cardiomyocytes (Fig. 1b). In conclusion, overexpression of 5-HT_{2A}

Fig. 1 Transient regulation of 5-HT_{2A} receptor expression in a mouse model of cardiac hypertrophy induced by aortic constriction. 8-week-old mice were subjected to transverse aortic constriction (TAC) or sham operation to trigger pressure-overload hypertrophy of the heart. **a** 5-HT_{2A} receptor levels were analyzed by immunoblotting on cardiac homogenates at 1 and 3 months after TAC. Immunoblots were performed on cardiac homogenates with anti-5-HT_{2A}, and GAPDH expression serves as a loading control. *Graph* represents densitometric analysis of 5-HT_{2A} receptor expression in sham or TAC hearts ($n = 3$). **b** 5-HT_{2A} receptor expression by immunohistochemistry in ventricles from sham or TAC hearts at 1 month ($n = 5$) ($\times 200$ magnitude). (* $p < 0.05$ vs. indicated value)



receptors in cardiomyocytes is transient and occurs during the early phase of hypertrophy.

M100907 treatment significantly reduced the development of pressure-overload hypertrophy

As we observed significant upregulation of 5-HT_{2A} receptors in cardiomyocytes during the development of hypertrophy, we evaluated the role of these receptors using a selective 5-HT_{2A} receptor antagonist, M100907. We treated mice with 0.1 mg/kg/day of M100907, a dose selective for 5-HT_{2A} receptors *in vivo* and maximally efficient in behavioral tests (Marek et al. 2005).

Cardiac morphology and function were analyzed by non-invasive echocardiography and invasive hemodynamics (Table 1). Following 2 months of TAC, significant increases in interventricular septum thickness (IVST) and posterior wall thickness (PWT) were observed in TAC-vehicle mice compared to sham-vehicle mice, which is a consequence of pressure-overload hypertrophy (Table 1). Interestingly, TAC-M100907 mice presented significantly lower IVST and PWT values compared to TAC-vehicle mice, indicating that hypertrophy was reduced. Administration of M100907 in sham mice did not alter any of the echocardiographic parameters. At 3 months, TAC-vehicle

mice had increased end-diastolic (EDD) and end-systolic diameters (ESD) compared to sham-vehicle mice, with preserved cardiac function since fractional shortening (FS%) was not significantly modified (Table 1). However, in TAC-M100907 mice at 3 months, cardiac dilatation was not observed (EDD and ESD) and FS was higher than in TAC-vehicle mice. Thus, M100907 treatment prevents the development of hypertrophy and limits the evolution to cardiac dilatation at a later stage.

Left ventricular function was assessed using a high-fidelity Millar catheter inserted through the cardiac apex. A significant decrease in the maximal left ventricular pressure was found following M100907 treatment, in both sham (-17.2 mmHg) and TAC (-21.1 mmHg) mice (Table 1). This drop in maximal pressure induced by 5-HT_{2A} blockade should cause a similar reduction in systolic arterial blood pressure. End-diastolic left ventricular pressure, dP/dt_{max} and dP/dt_{min} were not significantly modified, indicating that M100907 treatment did not affect left ventricular contractility or relaxation.

M100907 treatment significantly reduced other parameters associated with ventricle hypertrophy. Morphometric analysis in post-mortem animals demonstrated that heart/body weight (HW/BW) ratios were significantly lower in TAC-M100907 animals compared to TAC-vehicle animals

Table 1 Echocardiographic and hemodynamic parameters of aortic-banded mice

	Sham + vehicle	Sham + M100907	2 months		3 months	
			TAC + vehicle	TAC + M100907	TAC + vehicle	TAC + M100907
No. of animals	5	5	10	10	5	5
<i>Echocardiography</i>						
dPWT (mm)	0.62 ± 0.05	0.60 ± 0.03	0.91 ± 0.03*	0.75 ± 0.03*#	0.72 ± 0.07	0.82 ± 0.05*
dIVST (mm)	0.60 ± 0.03	0.54 ± 0.02	0.88 ± 0.04*	0.73 ± 0.04*#	0.76 ± 0.05	0.80 ± 0.04*
EDD (mm)	3.24 ± 0.04	3.26 ± 0.15	3.54 ± 0.13	3.57 ± 0.07*	3.74 ± 0.09*	3.30 ± 0.08#
ESD (mm)	1.76 ± 0.14	1.82 ± 0.11	2.11 ± 0.17	2.16 ± 0.08*	2.32 ± 0.01*	1.74 ± 0.17#
FS (%)	45.9 ± 3.9	44.5 ± 2.3	40.5 ± 2.2	39.9 ± 1.9	37.9 ± 1.3	47.5 ± 3.8#
HR (bpm)	582.2 ± 11.8	530.0 ± 31.7	586.5 ± 16.4	582.0 ± 14.4	532.5 ± 14.9*	597.8 ± 20.7#
<i>Hemodynamics</i>						
LVP _{max} (mmHg)	80.9 ± 3.9	63.7 ± 1.9#	107.0 ± 4.3*	86.0 ± 3.9*#	nd	nd
dP/dt _{max} (mmHg/s)	4,178 ± 207	3,494 ± 300	4,160 ± 348	3,536 ± 161	nd	nd
EDLVP (mmHg)	7.30 ± 1.58	8.59 ± 0.35	9.49 ± 1.06	7.22 ± 0.72	nd	nd
dP/dt _{min} (mmHg/s)	-4,515 ± 533	-3,482 ± 238	-4,665 ± 510	-3,803 ± 248	nd	nd

dPWT diastolic posterior wall thickness, dIVST diastolic interventricular septum thickness, EDD end-diastolic left ventricular diameter, ESD end-systolic left ventricular diameter, FS fractional shortening, HR heart rate, LVP_{max} maximal left ventricular pressure, EDLVP end-diastolic left ventricular pressure, dP/dt_{max} (dP/dt_{min}) maximal (minimal) first-order derivative of the LVP over time, nd not determined

* $p < 0.05$ versus corresponding sham mice; # $p < 0.05$ versus corresponding age-matched vehicle-treated mice

(Fig. 2a). Concerning ventricle/body weight ratios, the same difference was observed, albeit non-significant. Next, we performed specific measurements of cardiomyocyte cross-sectional areas on transverse sections of the hearts (Fig. 2b). Again, we found a significant reduction in cardiomyocyte size in TAC-M100907 treated mice compared to TAC-vehicle mice. Modification of genes involved in cardiac remodeling, such as foetal gene expression and fibrosis, was evaluated by real-time RT-PCR (Fig. 2c). In TAC animals, M100907 treatment decreased the expression of α SK-actin and β -MHC mRNA, compared to TAC-vehicle animals. Expression of ANP was not statistically different. In addition, expression of collagen I mRNA, but not collagen III mRNA, was decreased by M100907 treatment in TAC mice.

Taken together, our data show that 5-HT_{2A} selective antagonist M100907 prevents the development of pressure-overload ventricle remodeling in mice.

M100907 inhibits the activation of the CamKII–HDAC4 hypertrophic pathway

The CamKII pathway is a core mechanism that promotes myocardial hypertrophy and heart failure. CamKII is known to be activated by Gq-coupled receptors through autophosphorylation at Thr286 in response to elevation of Ca²⁺. Thus, we evaluated the activation of CamKII in hearts from TAC mice with a phospho-Thr286 antibody by Western blotting (Fig. 3). We found that hypertrophic hearts had enhanced levels of phospho-Thr286 CamKII

compared to sham hearts. Interestingly, the 5-HT_{2A} antagonist M100907 prevented the activation of CamKII during cardiac hypertrophy.

In the heart, CamKII is able to phosphorylate HDAC4 to promote pathological cardiac remodeling. Phosphorylation of HDAC4 on its serine residue by CamKII regulates its nucleocytoplasmic shuttling, relieving and derepressing hypertrophic gene expression. Thus, we evaluated HDAC4 phosphorylation with a phospho-Ser632 specific antibody in hearts from TAC and sham-operated animals (Fig. 3). We found that pressure overload increased the level of phospho-HDAC4. However, in M100907-treated animals, the phosphorylation of HDAC4 was inhibited. In conclusion, the 5-HT_{2A} selective antagonist M100907 prevents the activation of the CamKII–HDAC hypertrophic pathway in vivo.

Discussion

In the present study, we demonstrate that M100907, a selective 5-HT_{2A} receptor antagonist, prevents the development of myocardial hypertrophy in a model of pressure overload. Mice with experimental hypertrophy induced by TAC demonstrated reduced wall thickness, decreased cardiomyocyte area and reduced expression of genes involved in cardiac remodeling, when treated with M100907. In addition, 5-HT_{2A} receptor blockade inhibited the activation of the CamKII–HDAC4 pathway in the heart.

To our knowledge, this is the first study that addresses specifically the role of the 5-HT_{2A} receptor subtype in

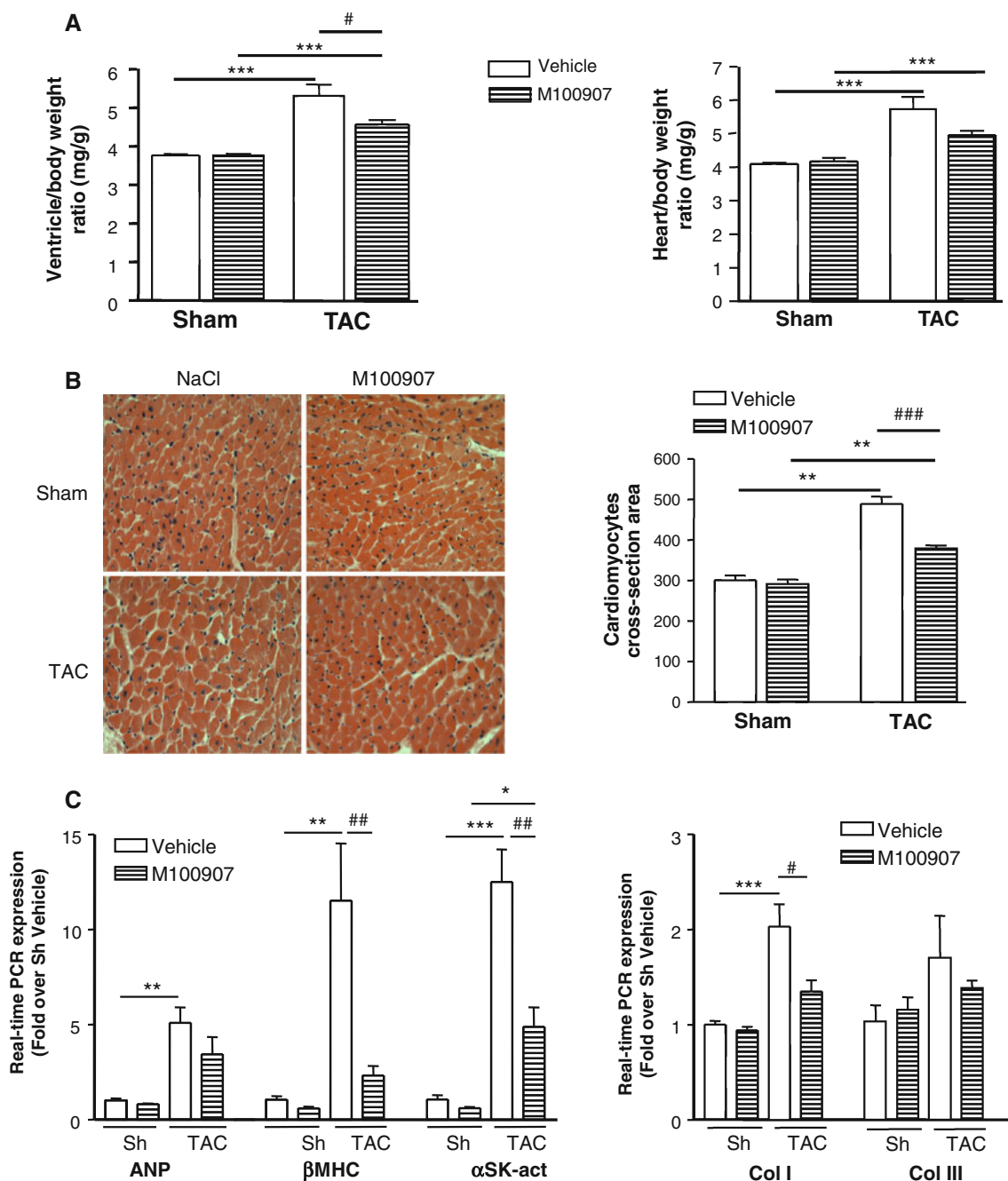


Fig. 2 M100907 prevents the development of cardiac hypertrophic parameters induced by TAC in mice. Mice were subjected to TAC or sham operation in the presence of 0.1 mg/kg/day of M100907 or vehicle, by osmotic mini-pumps. Hearts were collected 2 months after TAC for analysis. **a** Ventricle-to-body weight and heart-to-body weight ratios in sham ($n = 5$) and TAC ($n = 10$) vehicle-treated or M100907-treated mice. **b** Cardiomyocytes cross-sectional areas determined on hematoxylin–eosin stained sections using SigmaScan Pro (~200 cells/heart) with representative image on the left panel

($\times 400$ magnitude) and histogram on the right panel ($n = 5$ for sham and $n = 10$ for TAC mice). **c** Real-time PCR in ventricles from sham ($n = 5$) and TAC ($n = 10$) vehicle-treated or M100907-treated mice. Expression of the gene of interest is normalized to HPRT by the method of $2\Delta\Delta CT$, and results are expressed as fold over sham-vehicle mice ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$ vs. sham mice, $\#p < 0.05$; $##p < 0.01$; $###p < 0.001$ vs. TAC-vehicle-treated mice, 1-way analysis with post hoc Turkey test)

cardiac hypertrophy in vivo. Previous studies mainly focused on the 5-HT_{2B} receptor subtype, taking advantage of the availability of gene-targeted mice for this receptor. Indeed, authors nicely demonstrated that fibroblasts

5-HT_{2B} receptors played an important role in the secretion of pro-hypertrophic cytokines during cardiac remodeling (Jaffre et al. 2009; Monassier et al. 2008). In addition, pharmacological blockade of 5-HT_{2B} receptors with

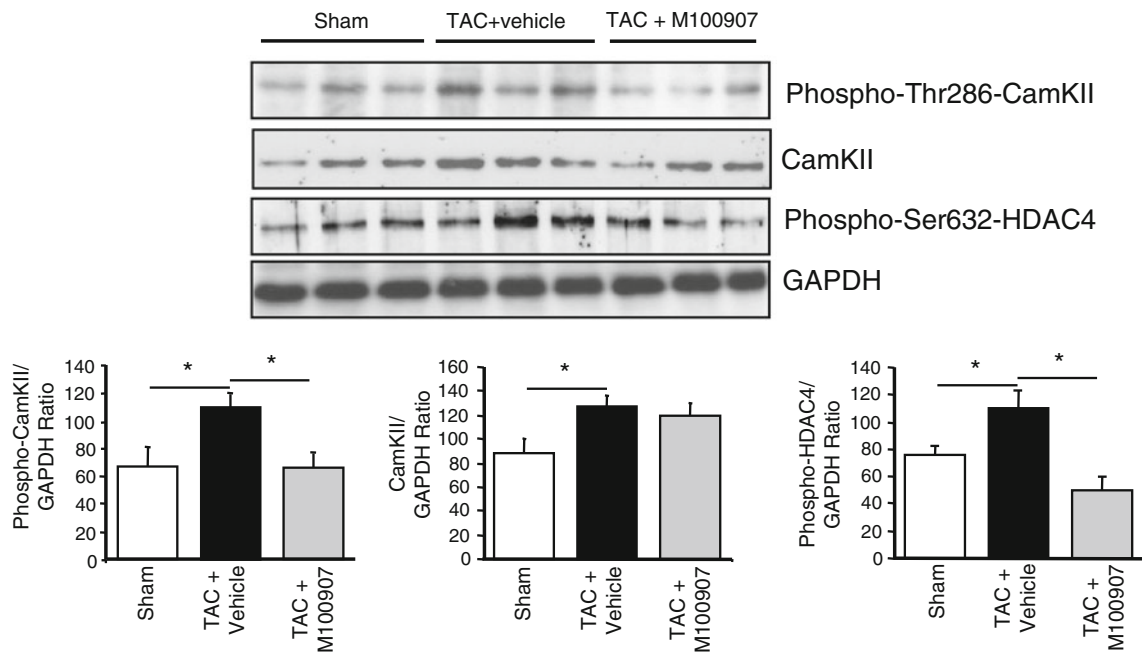


Fig. 3 M100907 attenuates TAC-induced activation of calcium-related proteins. Mice were subjected to TAC or sham operation in the presence of 0.1 mg/kg/day of M100907 or vehicle, by osmotic mini-pumps. Hearts were collected 3 months after TAC and heart homogenates were prepared for immunoblot analysis. Activation of CamKII and HDAC4 were evaluated with anti-phospho-Thr286–

CamKII and anti-phospho-Ser632–HDAC4 antibodies, respectively. Expression of CamKII was measured with anti-CamKII antibody. GAPDH serves as a loading control. Densitometric analysis were performed on $n = 5$ mice and were normalized to GAPDH as represented in the histogram. (* $p < 0.05$ vs. indicated value)

SB206553 or SB215505 confirmed their function in cardiac hypertrophy and failure in mice and rats (Jaffre et al. 2004; Liang et al. 2006; Monassier et al. 2008). Concerning the role of 5-HT_{2A} receptors in cardiac remodeling, some interesting findings have been published but they rely on indirect evidences or on the use of antagonists that lack specificity toward the 5-HT_{2A} receptor. Indeed, in a mouse model of enhanced 5-HT levels, hypertrophic response to pressure overload was exacerbated due to over-stimulation of 5-HT_{2A} receptors (Lairez et al. 2009). In addition, a number of studies demonstrated that the 5-HT_{2A} receptor antagonist ketanserin decreased cardiac hypertrophy in an animal model of hypertension (Xie et al. 2005) and in hypertensive patients (Cobo et al. 1990; Vyssoulis et al. 1990). However, ketanserin demonstrates combined blockade of 5-HT_{2A}/5-HT_{2B} receptors, along with high affinity for α 1-adrenergic receptors, which make it difficult to conclude on the specific role mediated by 5-HT_{2A} receptors. Another 5-HT_{2A} antagonist, sarpogrelate, showed beneficial effect in the model of ischemia–reperfusion and myocardial infarction in the rat, which supports a role for this receptor in cardioprotection (Rajesh et al. 2006; Sanganalmath et al. 2008). Here, we used M100907, a potent and selective 5-HT_{2A} receptor antagonist showing a >100-fold separation from activities at 5-HT_{2C} and >1000-fold separation with 5-HT_{2B} receptors, and we

provided evidence that 5-HT_{2A} receptors participate in the development of myocardial hypertrophy (Kehne et al. 1996; Knight et al. 2004).

Using immunohistochemistry in mouse heart, we observed that cardiomyocytes expressed 5-HT_{2A} receptors, as previously defined using radioligand binding assays (Jaffre et al. 2009). Interestingly, the increase in 5-HT_{2A} receptor expression observed in the early stage after pressure overload could indicate a transient role for 5-HT_{2A} in the initial development of hypertrophy, since 3 months after TAC, there was no upregulation of 5-HT_{2A} receptor in TAC heart. Our observations are supported by previous findings, showing that, in a rat model of hypertrophy induced either by pressure overload or by myocardial infarction, a 5-HT_{2A}-mediated inotropic response appeared in the heart, and that the response was strongly correlated with the degree of hypertrophy but not heart failure (Brattelid et al. 2007). At present, the mechanisms of regulation of 5-HT_{2A} receptors in cardiac hypertrophy are unknown.

The presence of 5-HT_{2A} receptors in cardiomyocytes and their role in mediating 5-HT-induced hypertrophic response in vitro (Mialet-Perez et al. 2012; Villeneuve et al. 2009) support a direct effect of M100907 inhibition on cardiac cells during pressure-overload hypertrophy. However, it is well known that 5-HT_{2A} receptor expression is not restricted to cardiomyocytes. 5-HT_{2A} receptors are expressed in

cardiac fibroblasts, where they participate in TGF β secretion and myofibroblasts transformation (Yabanoglu et al. 2009). Moreover, they are widely distributed among the body, in smooth muscle cells (where they participate in vasoconstriction) but also in platelets (where they mediate platelet aggregation) and in the central nervous system (where they induce sympatho-excitation) (Cote et al. 2004). Intraventricular hemodynamic parameters indicated that M10907 treatment did not affect cardiac contractility or diastolic function but induced, in both sham and TAC animals, a reduction in the maximal left ventricular pressure, which should result in a parallel drop in systolic arterial blood pressure. This phenomenon would thus probably participate in the reduction of myocardial hypertrophy evoked by M10907, by an indirect mechanism. Therefore, to determine the specific contribution of cardiomyocyte 5-HT_{2A} receptors among other cell types, cardiomyocyte-targeted KO mice will need to be developed.

The CamKII signaling pathway appears to be central to the pathological hypertrophic response in the heart (Anderson et al. 2011). CamKII is known to be activated by Gq-coupled receptors through autophosphorylation at Thr286 in response to sustained rise in intracellular Ca²⁺. CamKII activity is constantly increased in hypertrophied and failing myocardium from animal models and patients (Ling et al. 2009). In addition, inhibition of this Ca²⁺-sensitive kinase by drugs or gene deletion improves myocardial hypertrophy, which shows that CamKII constitute a central event during cardiac remodeling. Interestingly, in the present study, M10907 treatment significantly reduced CamKII Thr286 phosphorylation in cardiac homogenates from TAC mice. Thus, this decrease in CamKII activation likely contributes to the prevention of myocardial hypertrophy in M10907-treated TAC mice. In support of this, we found that one of the downstream signaling partners of CamKII, HDAC4, was also inhibited by M10907 treatment in TAC mice. HDAC4 is regulated by phosphorylation on Ser632 which induces the exclusion of this enzyme from the nucleus and allows the transcription of hypertrophic factors in the heart. In our model, HDAC4 phosphorylation was significantly reduced by M10907 treatment. This effect of 5-HT_{2A} receptor on activation of myocardial CamKII/HDAC4 signaling pathway has not been reported previously. Only in cultured cardiomyocytes, a link between 5-HT_{2A/B} receptors activation and HDAC5 export from the nucleus was demonstrated previously (Bush et al. 2004). Thus, our results bring new knowledge in the signaling mechanisms associated with 5-HT_{2A}-mediated hypertrophy.

In conclusion, this study demonstrates that M10907, an anti-psychotic drug with potential use in insomnia and depression, has beneficial effects on cardiac remodeling through inhibition of 5-HT_{2A}-mediated cardiac hypertrophy.

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