Cardiac overexpression of the human 5-HT₄ receptor in mice

Ulrich Gergs, Martin Baumann, Anne Böckler, Igor B. Buchwalow, Henning Ebelt, Larissa Fabritz, Steffen Hauptmann, Nicolas Keller, Paulus Kirchhof, Udo Klöckner, Klaus Pönicke, Uwe Rueckschloss, Wilhelm Schmitz, Franziska Werner, and Joachim Neumann

1Institut für Pharmakologie und Toxikologie, 2Institut für Pathologie, 3Julius-Bernstein-Institut für Physiologie, and 4Zentrum für Innere Medizin, Universitätshospital Tübingen, Tübingen, Germany

Submitted 23 July 2009; accepted in final form 6 July 2010

Cardiac overexpression of the human 5-HT₄ receptor in mice. Am J Physiol Heart Circ Physiol 299: H788–H798, 2010. First published July 16, 2010; doi:10.1152/ajpheart.00691.2009.—Serotonin (5-HT) exerts pleiotropic effects in the human cardiovascular system. Some of the effects are thought to be mediated via 5-HT₄ receptors, which are expressed in the human atrium and in ventricular tissue. However, a true animal model to study these receptors in more detail has been hitherto lacking. Therefore, we generated, for the first time, a transgenic (TG) mouse with cardiac myocyte-specific expression of the human 5-HT₄ receptor. RT-PCR and immunohistochemistry revealed expression of the receptor at the mRNA and protein levels. Stimulation of isolated cardiac preparations by isoproterenol increased phospholamban phosphorylation at Ser16 and Thr17 sites. 5-HT increased phosphorylation only in TG mice but not in wild-type (WT) mice. Furthermore, 5-HT increased contractility in isolated perfused hearts from TG mice but not WT mice. These effects of 5-HT could be blocked by the 5-HT₄ receptor-selective antagonist GR-125487. An intravenous infusion of 5-HT increased left ventricular contractility in TG mice but not in WT mice. Similarly, the increase in contractility by 5-HT in isolated cardiomyocytes from TG mice was accompanied by and probably mediated through an increase in L-type Ca²⁺ current and in Ca²⁺ transients. In intact animals, echocardiography revealed an inotropic and chronotropic effect of subcutaneously injected 5-HT in TG mice but not in WT mice. In isolated hearts from TG mice, spontaneous polymorphic atrial arrhythmias were noted. These findings demonstrate the functional expression of 5-HT₄ receptors in the heart of TG mice, and a potential proarrhythmic effect in the atrium. Therefore, 5-HT₄ receptor-expressing mice might be a useful model to mimic the human heart, where 5-HT₄ receptors are present and functional in the atrium and ventricle of the healthy and failing heart, and to investigate the influence of 5-HT in the development of cardiac arrhythmias and heart failure.

At present, seven groups of 5-HT-receptors have been distinguished (5-HT₁–5-HT₇) (29). The 5-HT₄ receptor mediates the positive inotropic effect in humans (8, 31, 33, 51). In the human atrium and ventricle, mRNAs of several splice variants of the 5-HT₄ receptor have been found (6, 2, 9).

In isolated multicellular preparations from human atria, 5-HT exerts a positive inotropic effect and a relaxant (or lusitropic) effect (31, 33). These effects were accompanied by increases in cAMP content and elevated PKA activity (32). In addition, in cardiomyocytes from human atria, 5-HT augmented current through L-type Ca²⁺ channels due to higher channel availability (24), phospholamban (PLB) phosphorylation (19), and enlarged cellular contractility (51). Activation of PKA is necessary for the 5-HT-mediated increase of L-type Ca²⁺ current in atrial cardiac myocytes (43). These effects were reversed by 5-HT₄ receptor-selective antagonists and, therefore, are regarded as 5-HT₄ receptor mediated. In isolated preparations from the human ventricle, only in preparations from some patients was a positive inotropic effect of 5-HT noted (8). The reason for this remains unclear. In the presence of phosphodiesterase inhibitors, consistently positive inotropic effects in human ventricular preparations were observed (8).

Intravenous injection of 5-HT exerts a positive chronotropic effect in humans (22). This effect may be 5-HT₄ receptor mediated because the increase of pacemaker current (a current implied to mediate positive chronotropic effects in the sinus node) by 5-HT in isolated cardiomyocytes from the human atrium is sensitive to the appropriate receptor blockers (46).

Moreover, 5-HT can induce atrial arrhythmias in isolated trabeculae from the human atrial myocardium (31). 5-HT may, e.g., thus initiate and/or sustain atrial fibrillation (AF) (27). In isolated human atrial cardiomyocytes, 5-HT prolonged action potentials and initiated late depolarizations (44). In the mouse heart, 5-HT₄ receptors seem to be expressed only during embryogenesis and disappear after birth (26). A cell culture model with adenal nor expression of human 5-HT₄ receptors in adult rat cardiac myocytes was established to explore receptor signaling in cardiomyocytes. This study (12) demonstrated coupling of transfected 5-HT₄ receptors to the cAMP pathway. So far, there have been no studies of the 5-HT₄ receptor in intact mice.

The present work was initiated to gain further insight into the cardiac effects of 5-HT. To this end, we generated, for the first time, a mouse with cardiac myocyte-directed expression of the human 5-HT₄ receptor. We hypothesized that in this model system, it would be possible to dissect in detail the physiological function of this receptor in the heart. Specifically, we wanted to know whether the overexpression of 5-HT₄ receptors

MOST OF THE SEROTONIN (5-HT) IN THE BLOOD ORIGINATES FROM ENTEROCROMAFFINE CELLS OF THE GASTROINTESTINAL TRACT (53). 5-HT IS RELEASED FROM THESE CELLS AND IS AVIDLY TAKEN UP BY PLATELETS. PLATELETS SEEM TO BE THE MAIN SOURCE OF 5-HT THAT INFLUENCES THE CARDIOVASCULAR SYSTEM. THESE EFFECTS INCLUDE VASOCONSTRICTION, AN INCREASE IN PLATELET AGGREGATION, APOTOPSIS OF CARDIAC CELLS, AUGMENTATION IN BEATING RATE, THE GENERATION OF ARRHhythmias (27), VALVULAR HEART DISEASE (49), AND POSITIVE INOTROPIC AND RELAXANT EFFECTS (FOR AN OVERVIEW, SEE REF. 29).
could lead to positive inotropic or chronotropic effects in the heart and/or arrhythmias.

MATERIALS AND METHODS

Isolation of 5-HT4a receptor cDNA and generation of transgenic mice. This investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). Animals were handled and maintained according to protocols approved by the Animal Welfare Committee of the University of Halle (approval no. 42502-02-691 MLK). Total RNA was extracted from the human heart using TRIzol reagent (Invitrogen) according to the manufacturer’s protocol. A 1,206-bp fragment encompassing 12 bp of the 5’-untranslated region, the entire 5-HT4a receptor-coding region, and 10 bp of the 3’-untranslated region was generated using RT-PCR methodology. The sequence was confirmed by sequencing. The RT-PCR product was ligated into a mouse cardiac α-myosin heavy chain promoter expression cassette containing the simian virus 40 (SV40) transcriptional terminator, similar to methods previously described (18). The transgene, which was composed of the α-myosin heavy chain promoter, the entire protein-coding region for the human 5-HT4a receptor, and the SV40 polyadenylation signal sequence, was isolated from the parent plasmid as a 7.1-kbp NruI fragment and used for the microinjection of fertilized mouse eggs (FVB/N). Transgene-positive mice were identified by Southern blot analysis and PCR assay of tail genomic DNA and thereafter crossed to the CD1 background.

RT-PCR analysis. Total RNA from mouse hearts was isolated as described above. Reverse transcription was performed with 4 μg RNA using random primers (Transcriptor High-Fidelity cDNA Synthesis Kit, Roche Applied Science, Mannheim, Germany), and PCR amplification was performed using Ampliqon Taq DNA polymerase (Bioulom, Hamburg, Germany) according to the manufacturer’s instructions. As a control, each sample was also analyzed without reverse transcription. As a positive control, a plasmid containing human 5-HT4a cDNA was used as a template. The following primer pair was used for the 5-HT4a receptor: forward 5’-ACATCTCTGAGCTCTGCCTG-3’ and reverse 5’-CCGACTGAGGCTGCTCCTG-3’ (product size: 439 bp).

Western blot analysis. Ventricular homogenates were prepared in 300 μl of 10 mM NaHCO3 and 100 μl of 20% SDS. Mixtures were kept at 25°C for 30 min before centrifugation to remove debris. Thereafter, supernatants (called homogenates) were kept at −20°C until further analysis. Western blot analysis was performed as previously reported (18). Aliquots of 100 μg protein were loaded per lane. Bands were detected using enhanced chemiluminescence (GE Healthcare, Munich, Germany) and a Storm PhosphorImager (GE Healthcare Biosciences). The Ca2+ concentration was gradually increased during digestion. After enzymatic digestion, hearts were perfused for 10 min with solution A, and ventricles were cut into several pieces before myocytes were separated by filtration through a nylon mesh.

Ca2+ transients and whole cell L-type Ca2+ currents. Measurement of Ca2+ transients were performed as previously described (18). Cells were incubated with the fluorescence dye Indo-1 and stimulated at 1 Hz. Excitation was at 365 nm, and the emitted fluorescence was recorded at 405 and 495 nm. The ratio of fluorescence at the two wavelengths was used as an index of cytosolic Ca2+.

Histological analyses. To analyze the morphological features of the transgenic (TG) myocardium, dewaxed and rehydrated tissue sections of formaldehyde-fixed ventricular samples were stained with hematoxylin and eosin (18).

Immunohistochemical techniques. For immunohistochemical analysis, sections of paraffin-embedded hearts were immunoreacted with antibodies to the 5-HT4a receptor in the same way as previously described (10, 18). Nuclei were counterstained with 4’,6-diamidino-2-phenylindole (Sigma). The controls were as follows: 1) omission of incubation with primary antibody, 2) substitution of primary antibody with rabbit IgG at the same final concentration, and 3) incubation in media containing primary antibody that had been preincubated with a 10-fold molar excess of the corresponding control peptide. Control incubations resulted in a lack of immunostaining. Images shown are representative of at least five independent experiments, which gave similar results.

Echocardiography. Echocardiography in spontaneously breathing mice was performed under anesthesia with 1.5% isoflurane as previously described using a 10MHz Toshiba ultrasound probe (14). Two-dimensional images and M-mode tracings were recorded from the parasternal short-axis view at the midpapillary level. Fractional shortening of the heart was calculated from the M-mode left ventricular (LV) diameters as follows: (LVEDD − LVESD)/LVEDD, where LVEDD is the LV-end-diastolic diameter and LVESD is LV-end-systolic diameter.

Histological analyses. To analyze the morphological features of the transgenic (TG) myocardium, dewaxed and rehydrated tissue sections of formaldehyde-fixed ventricular samples were stained with hematoxylin and eosin (18).

Echocardiography. Echocardiography in spontaneously breathing mice was performed under anesthesia with 1.5% isoflurane as previously described using a 10MHz Toshiba ultrasound probe (14). Two-dimensional images and M-mode tracings were recorded from the parasternal short-axis view at the midpapillary level. Fractional shortening of the heart was calculated from the M-mode left ventricular (LV) diameters as follows: (LVEDD − LVESD)/LVEDD, where LVEDD is the LV-end-diastolic diameter and LVESD is LV-end-systolic diameter.

Histological analyses. To analyze the morphological features of the transgenic (TG) myocardium, dewaxed and rehydrated tissue sections of formaldehyde-fixed ventricular samples were stained with hematoxylin and eosin (18).

Echocardiography. Echocardiography in spontaneously breathing mice was performed under anesthesia with 1.5% isoflurane as previously described using a 10MHz Toshiba ultrasound probe (14). Two-dimensional images and M-mode tracings were recorded from the parasternal short-axis view at the midpapillary level. Fractional shortening of the heart was calculated from the M-mode left ventricular (LV) diameters as follows: (LVEDD − LVESD)/LVEDD, where LVEDD is the LV-end-diastolic diameter and LVESD is LV-end-systolic diameter.

Histological analyses. To analyze the morphological features of the transgenic (TG) myocardium, dewaxed and rehydrated tissue sections of formaldehyde-fixed ventricular samples were stained with hematoxylin and eosin (18).
liquid nitrogen and kept frozen until further analysis. Briefly, 100 mg of frozen heart tissue were homogenized in 1 ml of 0.4 M HClO₄ containing 0.1% ascorbic acid, 0.1% cysteine, 3 mM glutathione, and 2 mM EDTA at 0°C. After centrifugation (5 min, 6,000 g, 0°C), 20 μl of the supernatant were neutralized with 3 μl of 2.5 M NaOH and directly injected into the HPLC column. HPLC separation was performed on a eurosphere 100–5C8 column (250 × 4.4 mm inner diameter, Knauer) at 30°C. 5-HT was eluted isocratically at a flow rate of 0.6 ml/min using a mobile phase consisting of methanol (200 ml) and 0.1 M KH₂PO₄ buffer (600 ml) adjusted to pH 4.5 (containing 170 mg 1-octanesulfonic acid and 60 mg EDTA per liter). Fluorescence detection was carried out at 345 nm with excitation at 285 nm.

Langendorff-perfused hearts. The heart was excised under anesthesia and retrogradely perfused on a vertical Langendorff apparatus, and monophasic action potentials were recorded from the right atrial epicardium and LV epicardium as previously described (16, 35). After an initial stabilization period, the intrinsic ventricular rhythm was observed after atrioventricular nodal block for 10 min to detect bradycardia-dependent arrhythmias. Thereafter, fix-frequent and programmed right atrial and ventricular stimulation was performed via an octapolar murine electrophysiology catheter. Action potential durations and arrhythmias were digitally analyzed using previously published computer-assisted techniques (21).

Data analysis. Data shown are means ± SE. Statistical significance was estimated by ANOVA followed by Bonferroni’s t-test or using a χ²-test as appropriate. P values of <0.05 were considered significant.

Drugs and materials. 1-[[2-[(methyl-sulphonyl) amino]ethyl]-4-piperdinyl-methyl-5-fluoro-2-methoxy-1H-indole-3-carboxylate (GR-125487) and 5-HT hydrochloride were obtained from Sigma. All other chemicals were of analytic grade. Deionized water was used throughout the experiments. Stock solutions were freshly prepared daily.

RESULTS

The human 5-HT₄a receptor was expressed under control of the α-myosin heavy chain promoter in cardiomyocytes of TG mice. With RT-PCR, a signal for the human 5-HT₄a receptor only was detected in mRNA isolated from the TG heart but not in mRNA from the wild-type (WT) heart (Fig. 1A). This indicates transcriptional activity of the transgene. In the same cDNA samples, no RT-PCR product for the mouse 5-HT₄ receptor was found (data not shown). 5-HT₄ receptor expression did not lead to cardiac hypertrophy over the time studied (Table 1), and the lifespan of TG mice was not different from WT mice. Expression of the transgene at the protein level was confirmed by immunohistochemistry (Fig. 1, B and C). The

![Fig. 1. Serotonin (5-HT₄) receptor expression in the hearts of adult (age: 12–14 wk) transgenic (TG) mice. A: RT-PCR analysis of total RNA isolated from the hearts of wild-type (WT) and TG mice revealed the expression of human 5-HT₄a receptor mRNA only in TG mice. Controls were omission of reverse transcription (RT–) and a plasmid as the template for PCR containing human 5-HT₄a receptor cDNA (Ctr). B: detection of 5-HT₄ receptors (green) in heart sections via immunohistology. Note the sarcolemmal localization of the signal corresponding to the receptor and its overexpression in sections from TG mice. C: higher magnification demonstrating the striated pattern of expression consistent with localization of the 5-HT₄a receptor with the t-tubule system.](http://ajpheart.physiology.org/)
green signal specific for the 5-HT4 receptor showed a striated pattern of expression, consistent with localization of the 5-HT4 receptor within the t-tubule system (Fig. 1C). In addition, the signal was prominent in the disci intercalares, consistent with sarcolemmal expression of the transgene. Control incubations, as stated in MATERIALS AND METHODS, resulted in a lack of sarcolemmal expression of the transgene. Immunostaining. We did not detect any fibrosis in animals using appropriate staining or other microscopic changes in morphology (data not shown). Next, we studied if overexpression of the 5-HT4 receptor altered the expression of important Ca2+-regulatory proteins in TG mice: protein expressions of PLB, SERCA, CSQ, TRD, and JCN remained unchanged (Fig. 2). The ratios (TG/WT) of the mean expression levels were as follows: SERCA, 1.13; CSQ, 1.05; TRD, 1.03; JCN, 0.90; and PLB, 0.94 (n = 3–6). The phosphorylation state of PLB at Ser16 was enhanced by isoproterenol in cardiomyocytes from both TG and WT mice (Fig. 3A). However, 5-HT enhanced PLB phosphorylation only in cardiomyocytes from TG mice and not from WT mice (Fig. 3A). This effect was blocked by the 5-HT4 antagonist GR-125487 (Fig. 3B). In a similar way, in isolated work-performing hearts, isoproterenol increased the phosphorylation of PLB at Ser16 (and Thr17) in TG and WT mice. However, 5-HT enhanced the phosphorylation of PLB at Ser16 (and Thr17) only in perfused hearts from TG mice but not from WT mice (Fig. 4A). Moreover, the higher basal Thr17 phosphorylation of PLB in TG mice was remarkable (Fig. 4B). In the presence of the 5-HT4 receptor-selective antagonist GR-125487 (1 μM), 5-HT-induced (1 μM) phosphorylation of PLB at Ser16 or Thr17 amounted to 109% or 105% respectively (n = 3 each), of the control value (no drug addition) in perfused hearts from TG mice. Hence, the effects of exogenously added 5-HT (1 μM) on dual PLB phosphorylation can tentatively be regarded as being 5-HT4 receptor mediated. Moreover, with GR-125487 (1 μM) alone, phosphorylation of PLB at Ser16 or Thr17 amounted to 108% or 107% ± 12%, respectively (n = 3), in perfused hearts from TG mice. The latter data are shown in original autoradiograms in Fig. 4B, namely, that the enhanced basal phosphorylation state of Thr17 on PLB in TG mice can be reduced to control values by perfusion with a 5-HT4 receptor antagonist. This indicates that the enhanced basal PLB Thr17 phosphorylation in perfused hearts is 5-HT4 receptor mediated, presumably due to endogenous 5-HT acting via this receptor (Fig. 4B).

Recently, the ability of neonatal rat cardiac myocytes to synthesize 5-HT has been reported (23). Therefore, one can ask whether overexpression of a 5-HT4 receptor changes the concentrations of 5-HT in heart tissue or plasma. As blood contains high levels of 5-HT, experiments were performed in isolated spontaneously beating hearts, where blood was washed out by retrograde perfusion with saline buffer. In tissue samples from

<table>
<thead>
<tr>
<th>Table 1. Gravimetric and HPLC determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>WT Mice</strong></td>
</tr>
<tr>
<td><strong>TG Mice</strong></td>
</tr>
<tr>
<td>Body weight, g</td>
</tr>
<tr>
<td>Heart weight, mg</td>
</tr>
<tr>
<td>Heart weight-to-body weight ratio</td>
</tr>
<tr>
<td>Plasma</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Norepinephrine, pg/mg</td>
</tr>
<tr>
<td>Epinephrine, pg/mg</td>
</tr>
<tr>
<td>5-HT (heart), pg/mg</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10 mice. Body weights and heart weights from wild-type (WT) and transgenic (TG) mice were measured. Moreover, values of the catecholamines norepinephrine, epinephrine, and serotonin (5-HT) were determined in plasma and, as a comparison, in samples from isolated buffer-perfused hearts. *P < 0.05, TG vs. WT mice.

Fig. 2. Expression of cardiac proteins. Expression of important Ca2+-regulatory proteins of the sarcoplasmic reticulum was unchanged in TG hearts compared with WT hearts. SERCA, sarco(endo)plasmic reticulum Ca2+ ATPase; CSQ, calsequestrin; TRD, triadin (Ψ glycosylated form of TRD); JCN, junctin; PLB, phospholamban.

Fig. 3. PLB phosphorylation in homogenates from cardiomyocytes of WT (A) and TG mice (A and B) (age: 12–14 wk). Western blots were probed with antibodies specific for CSQ (as a loading control) and PLB phosphorylated at Ser16 (PLB-PS16). Cardiomyocytes were treated before being frozen with isoproterenol (Iso), 5-HT, or GR-125487 (GR) at the concentrations indicated. Note the increase in the phosphorylation of PLB by 5-HT in cells from TG mice; this effect was blocked by GR, a 5-HT4 receptor antagonist.
TG mice (Fig. 5 and Table 2). Moreover, 5-HT increased Ca\(^{2+}\) because isoproterenol augmented this current in both WT and TG mice (Fig. 5). Namely, 1 μM 5-HT increased the amplitude of Ca\(^{2+}\) transients only in cardiomyocytes from TG mice but not from WT mice (Table 2). However, isoproterenol-mediated stimulation of L-type Ca\(^{2+}\) current was increased in both WT and TG mice (Fig. 5).

Levels of norepinephrine were higher in plasma from TG mice than from WT mice. Levels of epinephrine were similar in plasma from WT and TG mice, consistent with postrest potentiation. As shown in the trace of Fig. 7A, one beat is missing, and, later, a larger beat is noted, consistent with postrest potentiation. As shown in the bottom trace of Fig. 7A, an additional contraction with lower amplitude of Ca\(^{2+}\) transients in cardiomyocytes from TG mice by 106.2 ± 21.1% (P < 0.05), whereas 5-HT was devoid of any effect on cardiomyocytes from WT mice. These data fit well with the contractile findings. In isolated electrically driven cardiomyocytes from TG mice, 5-HT increased contractility, but not in cardiomyocytes from WT mice (Table 2). Additionally, LV pressure in cannulated mice was elevated by the infusion of 5-HT in TG mice but not in WT mice. For instance, 5-HT increased -dP/dt by 28.7 ± 8.07% (P < 0.05) of the predrug value (n = 10) in LV of TG mice, whereas in WT mice 5-HT at the same dose was inactive (97.2 ± 5.92% of the predrug value). Likewise, echocardiographic parameters of contractility (fractional shortening) were increased by 5-HT in intact TG mice but not in WT mice (Table 3). This stimulatory effect of isoproterenol was comparable in WT and TG mice (Table 3).

Finally, the same findings could be repeated in isolated work-performing hearts devoid of humoral and neural control of cardiac function (Table 4). In these preparations, the positive inotropic and relaxant effects of 5-HT in TG mice were completely blocked after the application of the antagonist GR-125487 (Table 4). Interestingly, in isolated hearts, the basal beating rate was increased in TG mice compared with WT mice, and this increase was not influenced by GR-125487 (Table 4). However, this phenomenon was only noted in vitro (working heart preparations). In vivo (echocardiography), the basal heart rate was not different between TG and WT mice (Table 3).

Therefore, and because of the resistance to GR-125487, it is unlikely that this tachycardia was directly 5-HT\(_4\) receptor mediated. Thus, we can only speculate that the mechanism leading to tachycardia in vitro is compensated by neural or humoral regulation of the heart beat in vivo. As a control, some animals were pretreated with reserpine (5 mg/kg, 16 h before the experiment) (42). The ability of reserpine under these conditions to greatly deplete cardiac stores of catecholamines and 5-HT is shown in a representative HPLC experiment in Fig. 6. In isolated perfused hearts from reserpine-pretreated TG animals, the effects of 5-HT on the force of contraction were still present (with 1 μM 5-HT, the force of contraction amounted to 87.2 ± 11.8% of nonreserpine-treated TG hearts, n = 5, P > 0.05), indicating that efficacy of 5-HT was comparable. This argues against an indirect positive inotropic effect of 5-HT in hearts from TG animals via the release of catecholamines. This conclusion is consistent with our contractile effects of 5-HT in cardiomyocytes, which do not contain nerve cells or nerve endings as a source of catecholamines.

In addition, a higher propensity toward arrhythmias was noted in TG mice compared with WT mice (Fig. 7). Arrhythmias were not noted in any of the hearts from WT mice. The pressure tracings of TG hearts showed an absolute arrhythmia at the LV level (Fig. 7A). For instance, as shown in the top trace of Fig. 7A, one beat is missing, and, later, a larger beat is noted, consistent with postrest potentiation. As shown in the bottom trace of Fig. 7A, an additional contraction with lower size follows a normal beat. This may be a mechanical equivalent to a delayed afterdepolarization. In this set of experiments, none of the 13 WT hearts exhibited ventricular arrhythmias, whereas arrhythmias under basal conditions (before the addition of exogenous 5-HT), as shown in Fig. 7A, were seen in 7 of 13 TG hearts (P < 0.05). These arrhythmias were observed in LV pressure recordings. We also inspected the pressure recordings in the left atrium of these mice (Fig. 7A) and failed to detect arrhythmias. Regrettably, our setup did not allow us to measure pressure in the right atrium. Hence, we can only speculate that the mechanism leading to tachycardia in vitro is compensated by neural or humoral regulation of the heart beat in vivo. As a control, some animals were pretreated with reserpine (5 mg/kg, 16 h before the experiment) (42). The ability of reserpine under these conditions to greatly deplete cardiac stores of catecholamines and 5-HT is shown in a representative HPLC experiment in Fig. 6. In isolated perfused hearts from reserpine-pretreated TG animals, the effects of 5-HT on the force of contraction were still present (with 1 μM 5-HT, the force of contraction amounted to 87.2 ± 11.8% of nonreserpine-treated TG hearts, n = 5, P > 0.05), indicating that efficacy of 5-HT was comparable. This argues against an indirect positive inotropic effect of 5-HT in hearts from TG animals via the release of catecholamines. This conclusion is consistent with our contractile effects of 5-HT in cardiomyocytes, which do not contain nerve cells or nerve endings as a source of catecholamines.

In addition, a higher propensity toward arrhythmias was noted in TG mice compared with WT mice (Fig. 7). Arrhythmias were not noted in any of the hearts from WT mice. The pressure tracings of TG hearts showed an absolute arrhythmia at the LV level (Fig. 7A). For instance, as shown in the top trace of Fig. 7A, one beat is missing, and, later, a larger beat is noted, consistent with postrest potentiation. As shown in the bottom trace of Fig. 7A, an additional contraction with lower size follows a normal beat. This may be a mechanical equivalent to a delayed afterdepolarization. In this set of experiments, none of the 13 WT hearts exhibited ventricular arrhythmias, whereas arrhythmias under basal conditions (before the addition of exogenous 5-HT), as shown in Fig. 7A, were seen in 7 of 13 TG hearts (P < 0.05). These arrhythmias were observed in LV pressure recordings. We also inspected the pressure recordings in the left atrium of these mice (Fig. 7A) and failed to detect arrhythmias. Regrettably, our setup did not allow us to measure pressure in the right atrium. Hence, we can only speculate that the mechanism leading to tachycardia in vitro is compensated by neural or humoral regulation of the heart beat in vivo. As a control, some animals were pretreated with reserpine (5 mg/kg, 16 h before the experiment) (42). The ability of reserpine under these conditions to greatly deplete cardiac stores of catecholamines and 5-HT is shown in a representative HPLC experiment in Fig. 6. In isolated perfused hearts from reserpine-pretreated TG animals, the effects of 5-HT on the force of contraction were still present (with 1 μM 5-HT, the force of contraction amounted to 87.2 ± 11.8% of nonreserpine-treated TG hearts, n = 5, P > 0.05), indicating that efficacy of 5-HT was comparable. This argues against an indirect positive inotropic effect of 5-HT in hearts from TG animals via the release of catecholamines. This conclusion is consistent with our contractile effects of 5-HT in cardiomyocytes, which do not contain nerve cells or nerve endings as a source of catecholamines.

In addition, a higher propensity toward arrhythmias was noted in TG mice compared with WT mice (Fig. 7). Arrhythmias were not noted in any of the hearts from WT mice. The pressure tracings of TG hearts showed an absolute arrhythmia at the LV level (Fig. 7A). For instance, as shown in the top trace of Fig. 7A, one beat is missing, and, later, a larger beat is noted, consistent with postrest potentiation. As shown in the bottom trace of Fig. 7A, an additional contraction with lower size follows a normal beat. This may be a mechanical equivalent to a delayed afterdepolarization. In this set of experiments, none of the 13 WT hearts exhibited ventricular arrhythmias, whereas arrhythmias under basal conditions (before the addition of exogenous 5-HT), as shown in Fig. 7A, were seen in 7 of 13 TG hearts (P < 0.05). These arrhythmias were observed in LV pressure recordings. We also inspected the pressure recordings in the left atrium of these mice (Fig. 7A) and failed to detect arrhythmias. Regrettably, our setup did not allow us to measure pressure in the right atrium. Hence, we can only speculate that the mechanism leading to tachycardia in vitro is compensated by neural or humoral regulation of the heart beat in vivo. As a control, some animals were pretreated with reserpine (5 mg/kg, 16 h before the experiment) (42). The ability of reserpine under these conditions to greatly deplete cardiac stores of catecholamines and 5-HT is shown in a representative HPLC experiment in Fig. 6. In isolated perfused hearts from reserpine-pretreated TG animals, the effects of 5-HT on the force of contraction were still present (with 1 μM 5-HT, the force of contraction amounted to 87.2 ± 11.8% of nonreserpine-treated TG hearts, n = 5, P > 0.05), indicating that efficacy of 5-HT was comparable. This argues against an indirect positive inotropic effect of 5-HT in hearts from TG animals via the release of catecholamines. This conclusion is consistent with our contractile effects of 5-HT in cardiomyocytes, which do not contain nerve cells or nerve endings as a source of catecholamines.
mias in six (from six) preparations persisted \((P < 0.05)\). This argues that the arrhythmias in TG cardiac preparations under basal conditions are due to stimulation of the overexpressed 5-HT\(_4\) receptor by endogenous 5-HT. In isolated Langendorff-perfused hearts, atrial monophasic action potentials were recorded. Here, spontaneous atrial arrhythmias were observed in four of eight TG mouse hearts but in only one of eight WT mouse hearts \((P < 0.05)\) (Fig. 7B, in the absence of exogenous 5-HT). After the application of exogenous 5-HT to the hearts without spontaneous arrhythmias, two additional hearts developed arrhythmias, but in WT hearts no effect of 5-HT was detectable. This was consistent with our observations in isolated cells: in ventricular cardiomyocytes from TG mice but not from WT mice, Ca\(^{2+}\) transients exhibited spontaneous oscillating increases in cytosolic Ca\(^{2+}\) content after stimulation with 1 \(\mu\)M 5-HT \((P < 0.05, n = 6;\) Fig. 7C). Interestingly, in only one of six TG cells, spontaneous Ca\(^{2+}\) oscillations were observed after \(\beta\)-adrenergic stimulation with 1 \(\mu\)M isoproterenol. We speculate here that the spontaneous arrhythmias may be mediated via Ca\(^{2+}\)-calmodulin-dependent protein kinase. This speculation is supported by the finding that in hearts from TG mice the phosphorylation of PLB at Thr17 under basal conditions was higher than in hearts from WT mice \((P < 0.05;\) Fig. 4). Additionally, in the first preliminary experiments, an

Table 2. Contractile parameters and L-type Ca\(^{2+}\) currents of isolated cardiomyocytes from TG mice and WT littermates

<table>
<thead>
<tr>
<th></th>
<th>WT Mice</th>
<th></th>
<th></th>
<th>TG Mice</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Iso</td>
<td>5-HT</td>
<td>Basal</td>
<td>Iso</td>
<td>5-HT</td>
</tr>
<tr>
<td>Percent shortening</td>
<td>4.73 ± 0.42</td>
<td>10.3 ± 1.17†</td>
<td>5.38 ± 0.54</td>
<td>5.47 ± 0.44</td>
<td>11.5 ± 0.98†</td>
<td>11.5 ± 0.84†</td>
</tr>
<tr>
<td>L-type Ca(^{2+}) currents</td>
<td>10.2 ± 1.6</td>
<td>16.6 ± 2.8†</td>
<td>14.4 ± 3.0</td>
<td>7.3 ± 1.5</td>
<td>16.3 ± 3.3†</td>
<td>16.0 ± 2.0*†</td>
</tr>
<tr>
<td>Current density, pA/pF</td>
<td>6.6 ± 0.5</td>
<td>8.6 ± 0.7</td>
<td>5.8 ± 0.4</td>
<td>5.7 ± 0.7</td>
<td>6.2 ± 0.8</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>Half-time, ms</td>
<td>6.6 ± 0.5</td>
<td>8.6 ± 0.7</td>
<td>5.8 ± 0.4</td>
<td>5.7 ± 0.7</td>
<td>6.2 ± 0.8</td>
<td>6.3 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 3–5\) hearts and 2–5 myocytes/heart. Data were recorded under basal conditions and after \(\beta\)-adrenergic stimulation with 1 \(\mu\)M isoproterenol (Iso) or 1 \(\mu\)M 5-HT. *\(P < 0.05\), TG vs. WT mice; †\(P < 0.05\) vs. basal conditions.
inhibitor of Ca²⁺-calmodulin-dependent protein kinase (W7) suppressed spontaneous ventricular arrhythmias in five (of five) preparations from TG mice (P < 0.05).

DISCUSSION

Aspects of the model generated. We chose to express the human 5-HT₄ receptor in mice because 1) the 5-HT₄ receptor was the first to be cloned; 2) its pharmacological profile is well studied, e.g., in heterologous expression systems; and 3) it is one of two main isoforms of the 5-HT₄ receptor in the human heart (7), which might confer to this model additional clinical relevance. Based on the functional experiments reported here, we did not detect any functional expression of 5-HT₄ receptors in WT mice, whereas overexpression of the human 5-HT₄ receptor was functionally noticeable in TG mice. This conclusion is supported by our immunohistological experiments, even if it seems that in WT hearts low amounts of 5-HT₄ receptors were expressed. Nevertheless, we cannot rule out that in WT samples some unspecific binding of the antibody occurred. On the other hand, 5-HT₄ mRNA has been detected in the mouse atrium (39).

Signal transduction. The signal transduction pathway of the human 5-HT₄ receptor in heart of TG mice is, in all respects studied in the present report, equal to that of the human heart: its stimulation by 5-HT is accompanied by increased current through L-type Ca²⁺ channels in TG hearts, presumably via the activation of PKA. This increased current [probably via Ca²⁺-induced Ca²⁺ release from the sarcoplasmic reticulum (for a review, see Ref. 40)] led to the observed increase in Ca²⁺ transients in TG mice. Consistent with the activation of PKA, we detected 5-HT₄-induced increases in the phosphorylation of PLB at Ser¹⁶ (in isolated cardiomyocytes and perfused beating hearts). The fact that exogenous 5-HT in beating perfused hearts from TG mice increased Thr¹⁷ phosphorylation is indicative of Ca²⁺-induced activation of Ca²⁺-calmodulin-dependent protein kinase. The dual phosphorylation of PLB (at Ser¹⁶ and Thr¹⁷) is thought to explain, at least in greater part, the inotropic and relaxant effects of β-adrenergic stimulation (for a review, see Ref. 40) and, by analogy, would account for the similar mechanical responses to exogenous 5-HT in hearts from TG mice. The fact that the 5-HT₄ receptor-selective antagonist GR-125487 attenuated the 5-HT₄-induced phosphorylation of PLB at Ser¹⁶ or Thr¹⁷ is consistent with 5-HT₄ receptor-mediated PLB phosphorylation. This phosphorylation pattern is similar to our findings in human atrial preparations (19). The fact that basal Thr¹⁷ phosphorylation (in the absence of exogenous 5-HT) was increased in perfused hearts from TG mice and was reduced by GR-125487 to WT levels is indicative that spontaneous arrhythmias (see below) in this model may be due in part to the basal activation of Ca²⁺-calmodulin-dependent protein kinase in hearts from TG mice. It can be asked why we did not notice tachycardia under in vivo conditions (echocardiography; Table 3) but in isolated perfused hearts (Table 4). One would assume that endogenous 5-HT should stimulated pacemaker cells in the sinoatrial node in both experimental setups. At present, we can only speculate that under in vivo conditions (echocardiography; Table 3), humoral and neural pathways are active on pacemaker cells and antagonize a (presumed) action of 5-HT on these cells. Specifically, an increase of vagal activity would have such an inhibitory and compensatory effect.

Comparison with β₁-adrenoceptor-overexpressing mice. In contrast with β₁-adrenoceptor-overexpressing mice [which show fibrosis (15)], no histological alterations were noted in our TG mice. It is noteworthy that β₁-adrenoceptor overexpression presumably uses, at least in part, the same signal

---

Table 3. Echocardiography of TG and WT mice

<table>
<thead>
<tr>
<th></th>
<th>WT Mice</th>
<th></th>
<th>TG Mice</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ctr</td>
<td>Iso</td>
<td>5-HT</td>
<td>Ctr</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>483 ± 12</td>
<td>580 ± 14†</td>
<td>498 ± 19</td>
<td>466 ± 19</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>0.36 ± 0.02</td>
<td>0.56 ± 0.02†</td>
<td>0.44 ± 0.02</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>LV posterior wall thickness at systole, mm</td>
<td>1.23 ± 0.03</td>
<td>1.48 ± 0.07†</td>
<td>1.32 ± 0.03</td>
<td>1.25 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5–7 mice. Mice were untreated (Ctr) or received Iso or 5-HT as described in MATERIALS AND METHODS. Fractional shortening was calculated as follows: (LVEDD − LVESD)/LVEDD, where LVESD is left ventricular (LV) end-diastolic diameter and LVEDD is LV end-systolic diameter, as described in MATERIALS AND METHODS. *P < 0.05, TG vs. WT mice; †P < 0.05 vs. Ctr.

Table 4. Isolated working heart preparations of TG and WT mice

<table>
<thead>
<tr>
<th></th>
<th>Frequency, beats/min</th>
<th>LV Pressure, mmHg</th>
<th>+dP/dt, mmHg/s</th>
<th>−dP/dt, mmHg/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG hearts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctr</td>
<td>272 ± 11</td>
<td>71.3 ± 2.2</td>
<td>2,498 ± 173</td>
<td>−1,234 ± 90</td>
</tr>
<tr>
<td>Iso</td>
<td>404 ± 20†</td>
<td>102.5 ± 10.2†</td>
<td>5,679 ± 576†</td>
<td>−3,047 ± 277†</td>
</tr>
<tr>
<td>5-HT</td>
<td>289 ± 26</td>
<td>77.3 ± 2.5</td>
<td>2,825 ± 351</td>
<td>−1,619 ± 128</td>
</tr>
<tr>
<td>GR-125487</td>
<td>330 ± 20*</td>
<td>71.8 ± 3.9</td>
<td>2,293 ± 269</td>
<td>−1,346 ± 185</td>
</tr>
<tr>
<td>GR-125487 + 5-HT</td>
<td>385 ± 16†</td>
<td>115.2 ± 17†</td>
<td>6,328 ± 1,620†</td>
<td>−3,607 ± 890†</td>
</tr>
<tr>
<td>GR-125487 + 5-HT</td>
<td>420 ± 28*†</td>
<td>103.9 ± 11.5*†</td>
<td>5,299 ± 1,297*†</td>
<td>−2,611 ± 665*†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 13 hearts. The following contractile parameters were measured using the work-performing mode: LV pressure, the maximum rate of LV pressure development (+dP/dt), and the maximum rate of LV pressure decline (−dP/dt). Hearts were left untreated (Ctr) or received Iso, 5-HT, GR-125487, or GR-125487 + 5-HT, as described in MATERIALS AND METHODS. *P < 0.05, TG vs. WT mice; †P < 0.05 vs. Ctr.
transduction system as the 5-HT₄ receptor, namely, adenylyl cyclase. In contrast, β₁-adrenoceptor-overexpressing mice exhibit fibrosis, hypertrophy, and, ultimately, heart failure and a reduced lifespan (15). However, in β₂-adrenoceptor-overexpressing mice, no fibrosis was discernable (41). The different phenotypes of β₁-adrenoceptor versus β₂-adrenoceptor overexpression are not completely understood but can be tentatively explained by the fact that the β₂-adrenoceptor couples in part to other pathways than the β₁-adrenoceptor, for instance, to inhibitory G proteins. The same may be true for the 5-HT₄ receptor. For instance, 5-HT₄ receptors can couple via Gβγ to L-type Ca²⁺ channels and, in addition, to ion channels (11, 45), and they can activate G13 (47). However, one can argue that 5-HT₄a receptor-mediated increases (in contrast to 5-HT₄b receptor-mediated increases) in cAMP production (in transfected HEK-293 cells) were not PTX sensitive, and, therefore, these receptors were regarded as not to signal via PTX-sensitive G proteins (45). It remains to be shown whether the

Fig. 6. Typical HPLC recordings. Samples were prepared from the hearts of WT mice (age: 12–14 wk) and injected into the HPLC system. The voltage of the detector (ordinates) was proportional to the amount of organic material injected. Abscissas indicate the retention time. Arrows indicate the retention times of known compounds. Samples at the top were analyzed for norepinephrine or epinephrine. Samples at the bottom were assayed for 5-HT and tryptophane (its precursor). The amounts corresponding to the peaks are given in picograms.

Fig. 7. Arrhythmias. A: pressure tracings of hearts from TG mice (age: 12–14 wk; perfused in the work-performing mode) showed an absolute arrhythmia at the left ventricular (LV) level. For instance, the top trace shows that one beat is missing and, later, a larger beat is noted, consistent with postrest potentiation. The bottom trace shows that an additional contraction with lower size follows a normal beat. This may be the mechanical equivalent to a delayed afterdepolarization. B: example of an episode of atrial fibrillation recorded by a right atrial (RA) monophasic action potential (MAP), a LV MAP, and an organ bath ECG from an isolated, beating, Langendorff-perfused TG heart. No exogenous 5-HT was added. C: example of spontaneous Ca²⁺ oscillations during Ca²⁺ transient measurements in isolated ventricular, electrically driven (1 Hz) TG cardiomyocytes in the presence of 1 μM 5-HT.
effects of 5-HT in this mouse model are still present after treatment of TG mice with PTX (which would be the prediction).

Further uses of 5-HT₄ TG mouse models. It is of interest to note that in WT mice at the concentrations (up to 100 μM) tested, 5-HT did not affect any functional parameters tested in this study. For instance, 5-HT did not increase the force of contraction (or the beating rate) in hearts from WT mice. Hence, mice provide an excellent background to test structure-effect relationships of the 5-HT₄ receptor in vivo. For instance, one could test mutations (25) in the heart of receptor-overexpressing mice. In future studies, heart failure could be induced in our or related TG mice (e.g., via aortic banding) to scrutinize how this intervention affects 5-HT₄ receptor function: for instance, in rats with congestive heart failure, 5-HT₄ receptors have been found in the LV, but functional responsiveness to 5-HT was mediated through both 5-HT₂A and 5-HT₄ receptors (5). In hearts from normal rats, only atrial (not ventricular) responsiveness to 5-HT was reported, and it was mediated through 5-HT₂A receptors (36). In human ventricles (from nonfailing and failing hearts), 5-HT can increase the force of contraction via 5-HT₄ receptors (8). Therefore, TG mice may present an appropriate model system for the study of 5-HT₄ receptors in nonfailing and failing human hearts.

Elevated plasma norepinephrine. It is not readily apparent why norepinephrine values in plasma (but not cardiac tissue; Table 1) are higher in TG mice than in WT mice. It is unlikely that the mechanism of neural release or the metabolism of norepinephrine is altered via 5-HT₄ receptor overexpression. The promoter used (the α-myosin heavy chain promoter) is only active in myocytes and not in neural cells (52); therefore, an altered release mechanism from neurons due to additionally overexpressed 5-HT₄ receptors in the neurons themselves is not compatible with the literature. Thus, we can presently only speculate that norepinephrine elevation in plasma may result from some afferent nerve stimulation leading to a central compensatory (or adjunct) mechanism. However, we failed to detect any cardiac hypertrophy or fibrosis in these mice. The exact reason for the enhanced norepinephrine plasma levels remains to be elucidated.

Arrhythmias. We noted, under basal conditions and after stimulation by the addition of exogenous 5-HT, more arrhythmias in isolated cardiac preparations from TG mice than from WT mice. These arrhythmias included both ventricular and supraventricular arrhythmias. Regarding the underlying postreceptor mechanism, it is tempting to speculate that increases in free intracellular Ca²⁺ led to afterdepolarizations (4), possibly via Ca²⁺-dependent autoactivation of Ca²⁺-calmodulin-dependent protein kinase feeding into a vicious circle. The increased basal Thr¹⁷ phosphorylation of PLB noted in TG hearts, which was antagonized by the selective 5-HT₄ receptor antagonist GR-125487, is supportive of this hypothesis. There is a wealth of literature for the involvement of Ca²⁺-calmodulin-dependent protein kinase in the genesis of cardiac arrhythmias (for a review, see Ref. 1). However, additional mechanisms, such as the action of 5-HT₄ receptors on pacemaker current in cardiomyocytes, could play an additional role (44, 46). Reserpine (via extensive reduction of 5-HT levels in the heart) and a selective 5-HT₄ receptor antagonist (GR-125487) could significantly reduce the incidence of arrhythmias in cardiac preparations from TG mice. These findings are consistent with the hypothesis that endogenous 5-HT, acting on overexpressed (functionally active) 5-HT₄ receptors (in TG mice), might underlie the arrhythmias we noted under basal conditions.

Not only does 5-HT lead to arrhythmias in human hearts, but the propensity of 5-HT to induce arrhythmias was even more pronounced in atrial preparations from patients chronically treated with β-adrenoceptor blockers (31, 51). Interestingly, in AF, mRNA expression of 5-HT₄ receptors was found to be altered. However, at present, the results are somewhat inconclusive. While Grammer and coworkers (20) provided evidence for a decrease of 5-HT₄ receptor mRNA in atrial tissue from patients with AF, more recently increased 5-HT₁b receptor mRNA expression in chronic AF has been reported (38). In the very same study (38), the authors noted decreased mRNA expression of 5-HT₁b receptors in acute AF. Thus, while the details are controversial, these data support a role for 5-HT via 5-HT₄ receptors in the etiology of human arrhythmias.

Conceivable uses of the present model system and comparison with other models. The present model may be useful to test the safety of (novel) 5-HT₄ receptor (partial) agonists and to ascertain an ability of new 5-HT₄ receptor antagonists to stop arrhythmias. For instance, cisapride, a compound affecting the tonus of the gastrointestinal system and a partial agonist of human atrial 5-HT₄ receptors (32), was found to evoke tachyarrhythmias (3, 28). Our TG system might be useful for preclinical testing of newer similar compounds. In the past, others (37, 48) have successfully used instrumented pigs to study the antirhythmic effects of 5-HT₄ receptor antagonists. The incidence of 5-HT-induced arrhythmias was greatly enhanced with a concomitant application of phosphodiesterase 3 inhibitors, at least in vitro (8), and caution should thus be used when combining a phosphodiesterase inhibitory drug with a drug that exerts partial agonistic properties on 5-HT₄ receptors (30). Moreover, it has been suggested that 5-HT₄ receptor antagonists might be useful to suppress supraventricular and ventricular arrhythmias in patients treated with phosphodiesterase inhibitors. Consistent with this, there is evidence for a protective function of phosphodiesterase 3 against 5-HT-evoked effects in isolated preparations from the human atrium (17).

The main new findings of the present work are as follows. Here, we report the first mouse model for cardiac overexpres-
sion of the 5-HT$_4$ receptor. We demonstrated that overexpression of 5-HT$_4$ receptors in the mouse heart led to 5-HT-mediated phosphorylation of regulatory proteins, increased Ca$^{2+}$ channel currents, and increased intracellular Ca$^{2+}$ transients, which probably explain the positive inotropic effect of 5-HT in these hearts. Furthermore, we noted arrhythmias in these TG mice, probably due to Ca$^{2+}$ overload of the sarcoplasmic reticulum. This model might be useful to better understand the role of 5-HT$_4$ receptors in health and disease.

ACKNOWLEDGMENTS

The work was part of the medical theses of A. Böckler and M. Baumann. The technical assistance of C. Geisler and J. Wehde is greatly appreciated. The authors gratefully acknowledge the TRAM facility of the Interdisciplinary Center of Clinical Research Münster for the expertise in the generation of transgenic mice.

GRANTS

This work was supported by the Deutsche Forschungsgemeinschaft and by the Interdisciplinary Center of Clinical Research Münster.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


H797
5-HT4 RECEPTOR OVEREXPRESSION


