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Molecular and pharmacological characterization of serotonin 5-HT_{2A} and 5-HT_{2B} receptor subtypes in dog

Pascal Bonaventure*, Diane Nepomuceno, Kirsten Miller, Jingcai Chen, Chester Kuei, Fredrik Kamme, Da-Thao Tran, Timothy W. Lovenberg, Changlu Liu

Johnson & Johnson Pharmaceutical Research and Development, L.L.C., 3210 Merryfield Row, San Diego, CA 92121, USA

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Abstract

We report the cloning, molecular characterization, and pharmacological characterization of the canine 5-HT_{2A} and 5-HT_{2B} receptors. The canine and human 5-HT_{2A} receptors share 93% amino acid homology. The canine and human 5-HT_{2B} receptors are also highly conserved (87% homology) with the exception of the carboxyl termini where the canine protein is 62 amino acids shorter. Both the canine 5-HT_{2A} and 5-HT_{2B} receptors have high affinity for [^{3}H]5-HT (K_D=4.50 ± 0.89 nM and 3.10 ± 0.82 nM, respectively) and, in general, the pharmacology of these two receptors matches closely the pharmacology of their human homologs for the 19 serotonergic ligands tested. However, the functional response (Ca^{2+} mobilization) of the canine 5-HT_{2B} receptor to several agonists was weaker compared to the human 5-HT_{2B} receptor. Using quantitative reverse transcriptase polymerase chain reaction, a high expression level of canine 5-HT_{2A} receptor mRNA was detected in the brain and lower levels in peripheral tissues, whereas the highest levels of canine 5-HT_{2B} receptor mRNA were observed in lungs and smooth muscles. A significant level of canine 5-HT_{2B} receptor mRNA was detected in brain tissue. The availability of the full sequence and pharmacology of the canine 5-HT_{2A} and canine 5-HT_{2B} receptors provides useful information for the interpretation of previous and future pharmacological studies of 5-$HT_{2A/2B}$ ligands in dog.

Keywords: 5-HT_{2A} receptor; 5-HT_{2B} receptor; Canine; Radioligand binding; Cloning

1. Introduction

The physiological actions of serotonin (5-hydroxytryptamine, 5-HT) are mediated by 14 different receptor subtypes, all but one belonging to the class of G-protein coupled receptors (Hoyer et al., 2002). These receptors are divided into seven distinct classes (5-HT_1 to 5-HT_7) largely on the basis of their structural and operational characteristics. The molecular, pharmacological, and physiological characterization of these receptors helps to elucidate the importance of these receptors as therapeutic targets.

The 5-HT_2 receptor family comprises three subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (for recent review, see Leysen, 2004). The 5-HT_2 receptors are linked to the Gq family of G-proteins, which subsequently activate phospholipase C, induce phosphoinositide metabolism, and increase intracellular calcium concentration (Foguet et al., 1992; Jerman et al., 2001; Porter et al., 1999; Roth et al., 1998). Both 5-HT_{2A} and 5-HT_{2B} receptors are found in the central nervous system and periphery, whereas 5-HT_{2C} is restricted to the central nervous system (Hoyer et al., 2002; Leysen, 2004).

The 5-HT_{2A} receptors mediate contractile responses in many vascular smooth muscle preparations (Cohen et al., 1981). In addition, platelet aggregation and increased capillary permeability following exposure to 5-HT have been attributed to 5-HT_{2A} receptor-mediated functions (De Chaffoy De Courcelles et al., 1985). Centrally, 5-HT_{2A} receptors are principally located in the cortex, claustrum, basal ganglia, and in several brain stem nuclei (Fonseca et

* Corresponding author. Tel.: +1 858 784 3078; fax: +1 858 450 2040.
E-mail address: pbonave1@prdus.jnj.com (P. Bonaventure).

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al., 2001; Hall et al., 2000). 5-HT_{2A} receptors in the medial nucleus of the tractus solitarius presumably play a role in 5-HT-induced hypotension and bradycardia (Huang and Pickel, 2003). 5-HT_{2A} receptor activation stimulates hormone secretion (e.g., ACTH (adenocorticotropic hormone), corticosterone, oxytocin, renin, and prolactin) (Van De Kar et al., 2001). Moreover, 5-HT_{2A} receptor agonists mediate certain behavioral syndromes in vivo. Head twitching in rat and mice can be inhibited with selective 5-HT_{2A} antagonists (Schreiber et al., 1995). 5-HT_{2A} antagonism is a property of certain antipsychotics and antidepressant drugs (Roth et al., 1998).

Activation of 5-HT_{2B} receptor leads to fundic smooth muscle contraction (Foguet et al., 1992; Kursar et al., 1992). 5-HT_{2B} receptors are found throughout the human gastrointestinal tract where they mediate contractile response. 5-HT_{2B} receptor has also been detected in discrete brain nuclei (Bonaventure et al., 2002; Duxon et al., 1997). Thus far, the role of 5-HT_{2B} receptors in the brain remains unclear. Stimulation by 5-HT of 5-HT_{2B} receptors on endothelial cells of the cerebral arteries causes release of nitric oxide, leading to vascular relaxation (Schmuck et al., 1996). Hence stimulation of 5-HT_{2B} receptors on meningeal blood vessels could be a trigger for migraine headache, perhaps explaining the reported prophylactic effect of 5-HT_2 receptor antagonists. 5-HT_{2B} receptor activation may also be involved in the development of cardiac valvulopathy associated with norfenfluramine and other serotonergic medication (Rothman et al., 2000).

The 5-HT_{2A} and 5-HT_{2B} receptor subtypes have been cloned from several species. The 5-HT_{2A} receptors from hamster, human, monkey, mouse, pig, rat, and sheep all have a similar length of 471 amino acids; the 5-HT_{2B} receptors from human, mouse, and rat have a length of 481, 504, and 460 amino acids, respectively (for review, see Kroeze et al., 2002). Both the 5-HT_{2A} and 5-HT_{2B} genes have three exons. Species differences in the binding of certain ligands between human and rat 5-HT_{2A} receptors have been reported where ergolines appear to display higher affinity for the rat receptor than for the human receptor (Pazos et al., 1984). This is due to an amino acid variation ($Ser^{242}Ala$) in the fifth transmembrane domain, between the human (^{242}Ser) and rat (^{242}Ala) 5-HT_{2A} receptors (Roth et al., 1998). Also for the 5-HT_{2B} receptor, species differences in ligand binding to the human and rat receptors were reported with ergolines and certain atypical antipsychotics displaying higher affinity for the human 5-HT_{2B} receptor (Bonhaus et al., 1995; Wainscott et al., 1996).

The canine 5-HT_{2A} has been cloned (Masuda et al., 2004) but not pharmacologically characterized, whereas the canine 5-HT_{2B} has neither been cloned nor characterized. However, several in vitro and in vivo pharmacological studies with 5-HT_2 ligands have been performed in canine (for examples, see Bush, 1987; Prins et al., 2001; Shoji et al., 1990). The canine is also frequently used to assess physiological or toxicological liabilities of early drug candidates, particularly for cardiovascular assessments. Therefore, we have cloned both the canine 5-HT_{2A} and 5-HT_{2B} receptor subtypes to address possible species differences in pharmacology. The receptors were expressed in recombinant cell lines and their pharmacological characteristics were compared to the human 5-HT_{2A} and 5-HT_{2B} receptors using radioligand binding and Ca^{2+} mobilization assays. In addition, their tissue expression profiles were also investigated using quantitative reverse transcriptase polymerase chain reaction (RT-PCR). During this study, the cloning of the canine 5-HT_{2A} cDNA receptor has been published but no pharmacological characterization was presented.

2. Materials and methods

All the experimental protocols comply with the European Community guidelines for the use of experimental animals.

2.1. Cloning of the canine 5-HT_{2A} receptor cDNA coding region

A 500 bp of canine 5-HT_{2A} DNA sequence was identified from NCBI GenBank (Genbank accession no. Y16134) using human 5-HT_{2A} sequence as the query. The resulting canine 5-HT_{2A} sequence was used to design primers for cloning of the full length cDNA using rapid amplification of cDNA end (RACE) method using canine brain cDNA as the template, which was synthesized from RNA extracted from Beagle dog (Bioreclamation Inc., Hicksville, NY) using a RACE cDNA kit from BD Biosciences (Palo Alto, CA). Canine 5-HT_{2A} gene-specific primer 1 (5′ TCT AGC GAG ATG GCG CAC AGG TGC ATG ATG 3′) was used for the 5′ end RACE. Canine 5-HT_{2A} gene-specific primer 2 (5′ CCA CCT TGT GTG TGA GTG ATC CTG GCA CAC3′) was used for the 3′ end RACE. The resulting cDNA was sequenced to obtain the complete coding region for the canine 5-HT_{2A}. Two primers (forward primer: 5′ ACT AGA CTC GAG GCC ACC ATG GAT GTC CTC TTT GAG GAT AAT GCT 3′ and reverse primer: 5′ ACT AGA GCG GCC GCT CAC ACA CAG CTA ACC TTT TCA TTC ACT GT 3′) were used to amplify the full length canine 5-HT_{2A} cDNA from the canine brain cDNA. The resulting 5-HT_{2A} cDNA full length was cloned into an eucaryotic expression vector pCIneo (Promega, Madison, WI) and the insert region was sequenced to confirm the sequence identity.

2.2. Cloning of the canine 5-HT_{2B} receptor cDNA coding region

A canine 5-HT_{2B} gene sequence was identified from the dog genome sequence (Genbank accession no. AAEX01019724) using the human 5-HT_{2B} DNA sequence as the query. Two primers (forward primer: 5′ AGT AGA

GAA TTC GCC ACC ATG GCC ATC TCT TAT AGA ATA TCA GAA C 3′ and reverse primer 5′ ACT AGA GCG GCC GCA TTA GGT GAA TAC CTC TAT TCC TTC TA 3′) were then designed according to the canine $5HT_{2B}$ genomic sequence to amplify the canine 5-HT_{2B} cDNA using canine brain cDNA as the template. The resulting DNA was cloned into a pCIneo vector and the insert region was sequenced to confirm the sequence identity.

2.3. Transient transfection and radioligand binding studies

COS-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) with 10% fetal bovine serum and transfected with the canine 5-HT_{2A} and 5-HT_{2B} receptor cDNA plasmids, respectively, using Fugene (Roche, Indianapolis) as described by the manufacturer. Two days after transfection, the COS-7 cells were detached with phosphate-buffered saline plus 5 mM EDTA and centrifuged at 1000 rpm for 5 min. The pellets were stored at − 80 °C. Membranes were homogenized in 50 mM Tris and 1 mM EDTA, and centrifugated at $20{,}000 \times g$ for 25 min. The resulting pellets were resuspended in 50 mM Tris, 0.5 mM EDTA, 5 mM $MgCl_2$, pH 7.4 (Roth et al., 2000) and aliquoted into 96-well plates (Greiner Bio-One, Frickenhausen, Germany). Nonspecific binding of the radioligands was estimated in the presence of 1 μM risperidone for both 5-HT_{2A} and 5-HT_{2B}. For ligand concentration binding isotherms, 10–12 concentrations of the radioligand ([^{3}H]5-HT), in a range of 0.1–20 nM, were used. Experiments were repeated independently at least three times. For inhibition of radioligand binding (1 nM [^{3}H]ketanserin for 5-HT_{2A} or 4 nM [^{3}H]mesulergine for 5-HT_{2B}), compounds were added at seven concentrations ranging from 0.01 nM to 10 μM.

Incubation of the membranes with the radioligands was run for 60 min at room temperature in a volume of 200 μl and then harvested by filtration through the GF/B filters (Packard, Meriden, CT) pretreated with 0.3% polyethyleneimine. The filters were washed five times with ice-cold buffer and dried in a 50 °C oven. Thirty-five microliters of Microscint 0 (Packard) was added to each well, and the plates were then counted on a Microscintillation counter (TopCount NTX, Packard, Meriden, CT). Ligand concentration binding isotherms and sigmoidal inhibition curves were generated and fitted using nonlinear regression analysis (GraphPad Prism software, San Diego, CA). The B_{max} and apparent K_D values of the radioligands and pIC_{50} of the inhibitor were free parameters for the curve fitting. Apparent K_i values were calculated as $K_i = IC_{50}/(1 + C/K_D)$, where C is concentration of the radioligand and $pK_i = -\log K_i$. Data are expressed as mean ± S.E.M. Final protein content was assayed according to the method of BCA (bicinchoninic acid) protein assay kit (Pierre, Rockford, IL).

Human 5-HT_{2A} and 5-HT_{2B} receptors were included for comparison. NIH 3T3 cells stably expressing the recombinant human 5-HT_{2A} receptor were grown in DMEM with 10% fetal bovine serum, 1% penicillin–streptomycin, and 600 μg/ml G418. CHO cells stably expressing the recombinant human 5-HT_{2B} receptor were grown in DMEM/F-12 Nutrient Mixture supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, and 400 μg/ml G418.

2.4. Transient transfection and Ca^{2+} mobilization assays

HEK-293 cells were cultured in DMEM with 10% fetal bovine serum, 1% Penn–Strep, 10 mM HEPES, and sodium pyruvate in 10 cm tissue culture dishes. Canine 5-HT_{2A} and 5-HT_{2B} cDNAs were transiently transfected into HEK-293 cells using Lipofectamine (Invitrogen, Carlsbad, CA). Forty-eight hours after transfection, the cells were detached with phosphate-buffered saline plus 10 mM EDTA, washed with cold serum-free DMEM/F-12, and dye-loaded with 4 μM Ca^{2+} dye Fluo-3AM in serum-free DMEM/F-12 with 2.5 mM probenecid. Dye-loaded cells were plated on to 96-well ViewPlates (Packard, Meriden, CT) and incubated at 37 °C, 5% CO_2 for 1 h. Once dye-loaded, dye media was replaced with serum-free DMEM/F-12. For antagonist potency determinations, cells were pre-incubated with compounds (diluted in DMEM/F-12) for 10 min before agonist stimulation. Ligand-induced Ca^{2+} release was measured using a Fluorometric Imaging Plate Reader (FLIPR; Molecular Devices, Sunnyvale, CA). Functional responses were measured as peak fluorescence intensity minus basal. The concentration of agonist that produced a half-maximal response is represented by the EC_{50} value. Relative efficacy values are the corresponding fraction of the response elicited by the compounds compared to 10 μM 5-HT. Antagonistic potency values were converted to apparent pK_B values using a modified Cheng–Prusoff correction. Apparent $pK_B = -\log IC_{50}/1 + [\text{concentration of agonist}/EC_{50}]$. Data are expressed as mean ± S.E.M.

2.5. Quantitative RT-PCR for 5-HT_{2A} and 5-HT_{2B} mRNA detection in canine tissues

Total RNA from different dog tissues (Beagle, Bioreclamation Inc., Hicksville, NY) was isolated using Trizol (Invitrogen) and treated with DNase I (Epicentre Technologies, Madison, WI) to remove genomic DNA. cDNA was synthesized from 3 μg of total RNA of each tissue using SuperScript III RT (Invitrogen) with 100 ng of oligo $dT_{18–21}$ (Amersham Biosciences, UK). The reaction was incubated at 50 °C for 30 min then heat-inactivated at 80 °C for 3 min and chilled on ice. The cDNA was diluted 30-fold and 2 μl of each sample was analyzed by quantitative PCR using the SmartCycler (Cepheid, Sunnyvale, CA) in quadruplicates. The PCR mix consisted of 0.2× Sybr Green I (Invitrogen), 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 1 U of TaqStart Antibody (Becton Dickinson, Palo Alto, CA), 3 U of AmpliTaq DNA

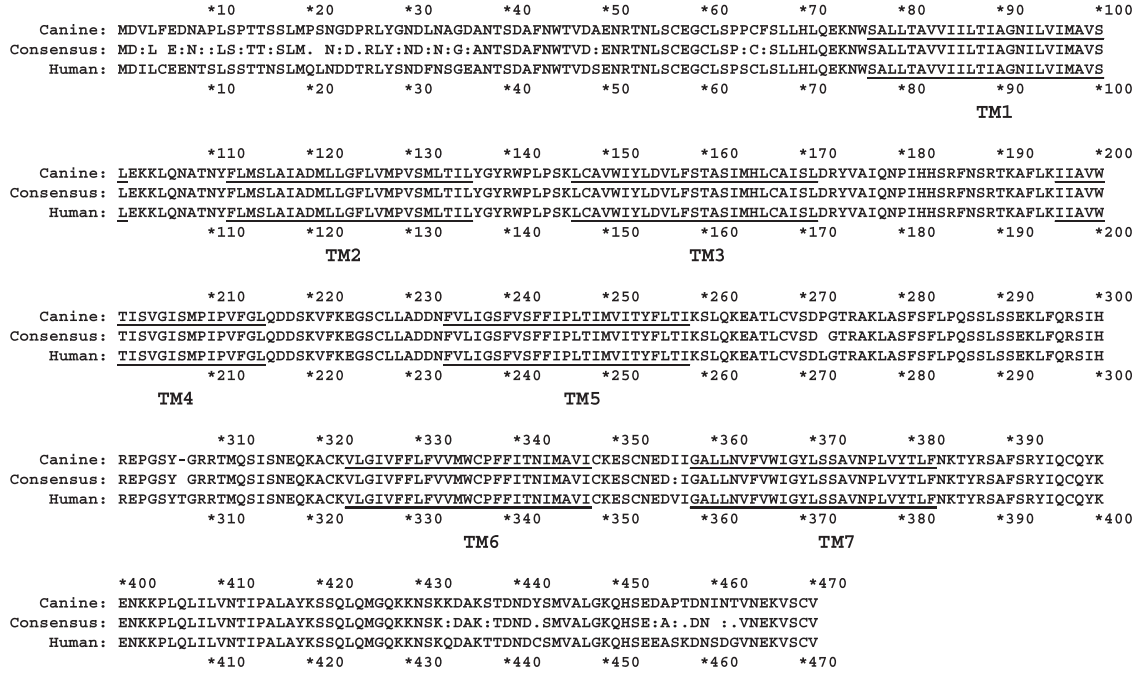

Fig. 1. Amino acid sequence comparison of human and canine 5-HT_{2A} receptors. The consensus sequence is also shown. The putative seven transmembrane domains are indicated with solid line.

polymerase, 200 mM Trehalose (Sigma), and 200 μM dNTPs (Amersham). PCR primers were from GenBase (San Diego, CA). Standard curves were generated for each gene by dilution of linearized plasmids containing an insert for the gene of interest (canine 5-HT_{2A} forward: TGGATCGGTTACCTCTCCTC reverse: GGCCGGTATAGTGTTCACTA; canine 5-HT_{2B} forward: ACAGTGGGCAGCTCTTCTGA reverse: CCAACTAGCAGATCAGCCAC), or of PCR products generated by a primer pair straddling the primer pair used for quantitation (β-actin). PCR cycle parameters were: initial hold at 95 °C for 90 s, 40 cycles of 95 °C for 5 s, 62 – 70 °C (depending on primer pair) for 7 s, and 72 °C for 15 s. At the end, a melt curve analysis was performed.

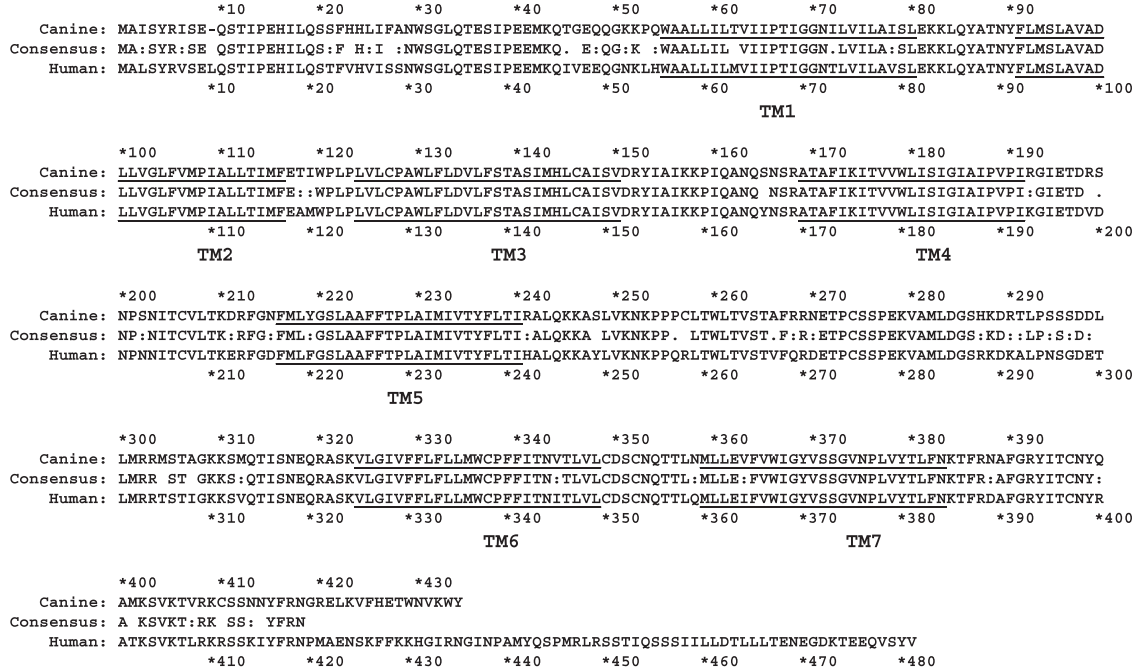

Fig. 2. Amino acid sequence comparison of human and canine 5-HT_{2B} receptors. The consensus sequence is also shown. The putative seven transmembrane domains are indicated with solid line.

2.6. Materials

[^{3}H]5-HT (30.0 Ci/mmol) and [^{3}H]ketanserin (76.5 Ci/mmol) were obtained from PerkinElmer Life Science (Boston, MA) and [^{3}H]mesulergine (97 Ci/mmol) was obtained from Amersham Bioscience (UK). Risperidone, ritanserin, ketanserin, olanzapine, eplivanserin, and MDL-100907 were obtained through the compound logistic center of Johnson & Johnson Pharmaceutical Research and Development, LLC. SB-204741 (*N*-(1-methyl-5-in-dolyl)-*N*′-(3-methyl-5-isothiazolyl) urea), 5-HT (5-hydroxytryptamine), 5-CT (5-carboxyamidotryptamine), α-Me-5-HT (2-methyl-5-hydroxytryptamine), mesulergine, yohimbine, and methiothepin were obtained from Sigma RBI (St. Louis, MO). BW-723C86 (1-{5-(2-thienyl-methoxy)-1*H*-3-indolyl}propan-2-amine), *m*-CPP (1-(3-chlorophenyl)piperazine, DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, RU-24969 (5-methoxy-3(1,2,3,6-tetrahydro-4-pyridiny)-1*H*-indole), SCH-23390 (*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro(1*H*)-3-benzapine), and TFMPP (*N*-(3-trifluoromethyl-henyl)piperazine were obtained from Tocris Cookson, (Bristol, UK). All cell culture reagents were obtained from Hyclone (Logan, UT).

3. Results

3.1. Cloning of canine 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor cDNA

We initially identified a 500 bp fragment of canine 5-HT$_{2A}$ DNA sequence from NCBI GenBank (accession no. Y16134) using human 5-HT$_{2A}$ sequence as the query. The resulting canine 5-HT$_{2A}$ sequence was used to design primers for cloning of the full length cDNA using the RACE method. The resulting canine 5-HT$_{2A}$ cDNA full length sequence was assembled from the sequences derived from the RACE PCR products. cDNA containing the complete coding region of canine 5-HT$_{2A}$ was PCR-amplified from canine brain cDNA pool and cloned into pCIneo. The cloned cDNA was sequenced to confirm the sequence identity and the complete coding region was submitted to Genbank (accession no. AY832858). Our results showed that the canine 5-HT$_{2A}$ cDNA encodes 470 amino acids, which is one less than human 5-HT$_{2A}$. Amino acid sequence comparison between the canine and human 5-HT$_{2A}$ receptors showed that they share about 93% homology, indicating that 5-HT$_{2A}$ receptor is well conserved between canine and human (Fig. 1).

The canine 5-HT$_{2B}$ gene was identified from the canine genome draft sequence using the human 5-HT$_{2B}$ DNA sequence as the query. Two primers were then designed according to the canine 5-HT$_{2B}$ genomic sequence predicted to amplify the canine 5-HT$_{2B}$ cDNA coding sequence using canine brain cDNA as the template. The resulting DNA was cloned into pCIneo vector and the insert region was sequenced. The complete coding region of canine 5-HT$_{2B}$ was submitted to Genbank (accession no. AY832859). Sequencing results indicated that the canine 5-HT$_{2B}$ cDNA is highly conserved to the human 5-HT$_{2B}$ (87% homology) with the exception of the C-terminal region. While the human 5-HT$_{2B}$ has 481 amino acids, the canine 5-HT$_{2B}$ gene only encodes 434 amino acids, missing the C-terminal tail corresponding to the last 62 amino acids of the human 5-HT$_{2B}$ (Fig. 2). Using RACE technique, the canine 5-HT$_{2B}$ cDNA sequence was further investigated from two additional dogs from different sources and the same sequence was found.

3.2. Ligand binding profile of recombinant canine 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors

Saturation binding studies and Scatchard analysis performed over 10 concentrations of [^{3}H]5-HT (maximal concentration 20 nM) demonstrated that membranes

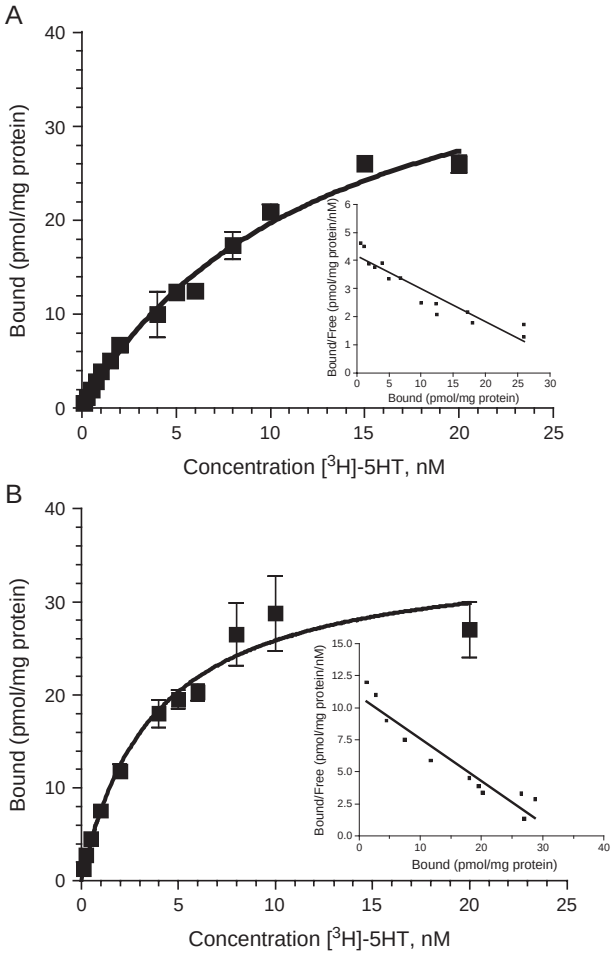

Fig. 3. Representative saturation binding isotherm of [^{3}H]5-HT binding to (A) canine 5-HT$_{2A}$/COS-7 membranes and (B) canine 5-HT$_{2B}$/COS-7 membranes. The non-specific binding was determined in the presence of 1 μM risperidone for both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors. Data points represent specific binding calculated by subtracting non-specific binding from total binding. Insert: Scatchard plot of the same data. The derived K_D and B_{max} are listed in Table 1. Data are mean ± S.E.M.

Table 1
Apparent equilibrium dissociation constant (K_D) and maximum number of binding sites (B_{max}) values measured with [^{3}H]5-HT for binding to canine and human 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors in recombinant cell membranes

	c5-HT$_{2A}$ COS-7	h5-HT$_{2A}$ NIH3T3	c5-HT$_{2B}$ COS-7	h5-HT$_{2B}$ CHO
K_D (nM)	4.50 ± 0.89	1.75 ± 0.17	3.10 ± 0.82	2.67 ± 0.54
B_{max} (pmol/mg protein)	25.80 ± 3.51	25.5 ± 4.58	30.75 ± 4.05	35.67 ± 7.12

Values are mean±S.E.M. (n=3–6).

obtained from COS-7 cell transiently transfected with the canine 5-HT$_{2A}$ or canine 5-HT$_{2B}$ showed a single population of high affinity binding sites for [^{3}H]5-HT (Fig. 3). K_D and B_{max} values are given in Table 1. Values for the human 5-HT$_{2A}$ and human 5-HT$_{2B}$ receptors are also given for comparison in Table 1 and were found to be within a similar range to the values obtained for the canine receptors. No detectable specific [^{3}H]5-HT binding was observed in nontransfected cell membranes.

The affinity constants (pK_i values) of 19 serotonergic compounds (9 putative agonists and 10 putative antagonists) for the displacement of [^{3}H]ketanserin (canine 5-HT$_{2A}$, K_D=1.3 ± 0.12 nM) or [^{3}H]mesulergine (canine 5-HT$_{2B}$, K_D=5.43 ± 0.69 nM) from membranes were obtained from COS-7 cells expressing canine 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors. Antagonist radioligands ([^{3}H]ketanserin and [^{3}H]mesulergine) were used for competition binding. Antagonists usually do not show a marked difference in binding affinity between coupled and uncoupled states of the 5-HT$_2$ receptor subtypes. Similar competition binding experiments were performed in parallel on membranes obtained from NIH3T3 or CHO cells expressing human 5-HT$_{2A}$ or human 5-HT$_{2B}$ receptors, respectively. Data are presented in Table 2. In Fig. 4, pK_i values of the 19 serotonergic compounds obtained with membranes from the cloned canine 5-HT$_{2A}$ or canine 5-HT$_{2B}$ receptors have been plotted against pK_i values obtained with the same compounds using membranes from cells expressing the cloned human 5-HT$_{2A}$ or human 5-HT$_{2B}$ receptors. The affinity constants at the canine 5-HT$_{2A}$ receptor showed a high correlation with those at the human 5-HT$_{2A}$ receptor (Fig. 4A). Similarly, affinity constants at the canine 5-HT$_{2B}$ receptor showed high correlation with those at the human 5-HT$_{2B}$ receptor (Fig. 4B). In contrast, the correlation at the canine 5-HT$_{2A}$ with canine 5-HT$_{2B}$ receptor was much weaker (Fig. 4C); similarly, a low correlation was obtained between the human 5-HT$_{2A}$ and human 5-HT$_{2B}$ (Fig. 4D).

Compounds known to display significant selectivity for human 5-HT$_{2A}$ receptor vs. human 5-HT$_{2B}$ receptor (risperidone, MDL-100907, eplivanserin, and ketanserin) were found to display a similar selectivity profile on the canine receptor subtypes (Table 2). Similarly, compounds

Table 2
Affinity constants (pK_i) of serotonergic ligands for inhibition of [^{3}H]ketanserin or [^{3}H]mesulergine binding to membranes derived from COS-7, CHO, or NIH3T3 cells expressing recombinant canine or human 5-HT$_{2A}$ or 5-HT$_{2B}$ receptors

Compound	c5-HT$_{2A}$ COS-7 [^{3}H]ketanserin	h5-HT$_{2A}$ NIH3T3 [^{3}H]mesulergine	c5-HT$_{2B}$ COS-7 [^{3}H]ketanserin	h5-HT$_{2B}$ CHO [^{3}H]mesulergine	Ratio c5-HT$_{2A}$ vs. h5-HT$_{2A}$	Ratio c5-HT$_{2B}$ vs. h5-HT$_{2B}$
Agonists						
5-HT	6.75 ± 0.11	6.96 ± 0.06	8.52 ± 0.08	8.35 ± 0.06	1.63	0.67
DOI	7.92 ± 0.12	8.29 ± 0.12	7.88 ± 0.15	7.82 ± 0.08	2.37	0.87
m-CPP	6.71 ± 0.01	7.06 ± 0.07	7.68 ± 0.05	7.54 ± 0.07	2.23	0.73
SCH-23390	7.79 ± 0.14	7.87 ± 0.12	6.51 ± 0.12	6.56 ± 0.04	1.21	1.12
α-Me-5-HT	6.76 ± 0.28	7.12 ± 0.11	8.54 ± 0.09	8.50 ± 0.09	2.31	0.91
BW-723C86	6.20 ± 0.19	6.41 ± 0.20	7.58 ± 0.06	8.11 ± 0.06	1.60	3.36
TFMPP	6.67 ± 0.12	7.06 ± 0.11	7.38 ± 0.09	7.40 ± 0.05	2.45	1.04
RU-24969	5.90 ± 0.14	6.00 ± 0.14	7.89 ± 0.41	7.61 ± 0.07	1.26	0.53
5-CT	5.64 ± 0.07	5.94 ± 0.04	7.13 ± 0.07	7.21 ± 0.08	1.99	1.20
Antagonists						
Ritanserin	8.12 ± 0.06	8.49 ± 0.06	8.40 ± 0.01	8.34 ± 0.07	2.33	0.87
Risperidone	8.50 ± 0.13	9.04 ± 0.16	7.35 ± 0.24	7.59 ± 0.05	3.54	1.73
MDL-100907	8.76 ± 0.06	9.12 ± 0.01	5.90 ± 0.05	5.96 ± 0.05	2.32	1.16
Eplivanserin	9.00 ± 0.16	9.70 ± 0.08	5.72 ± 0.04	5.85 ± 0.06	4.99	1.36
SB-204741	<5	<5	6.74 ± 0.08	6.92 ± 0.01	1.00	1.50
Mesulergine	7.04 ± 0.14	7.64 ± 0.14	7.77 ± 0.15	8.06 ± 0.06	4.01	1.92
Yohimbine	5.01 ± 0.01	5.58 ± 0.01	7.09 ± 0.12	7.18 ± 0.07	3.68	1.25
Methiothepin	8.45 ± 0.17	8.98 ± 0.08	8.12 ± 0.16	8.38 ± 0.03	3.42	1.82
Ketanserin	7.84 ± 0.09	8.54 ± 0.16	6.20 ± 0.14	6.38 ± 0.14	4.95	1.51
Olanzapine	7.97 ± 0.15	8.49 ± 0.19	7.46 ± 0.10	7.97 ± 0.11	3.30	3.24

pK_i values are the mean ± S.E.M of three to eight independent experiments.

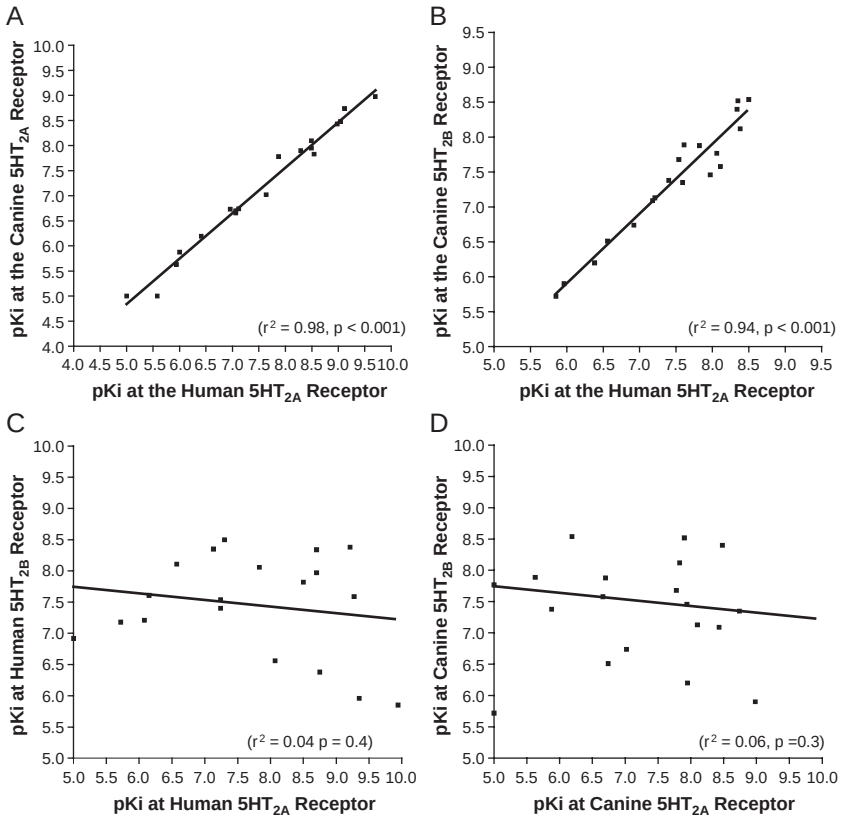

Fig. 4. Regression analysis of binding affinity constants (pK_i values) of serotonergic ligands (see Table 2) at the cloned canine 5-HT_{2A} and 5-HT_{2B} receptors with human 5-HT_{2A} and 5-HT_{2B} receptors. (A) Comparison of canine and human 5-HT_{2A} binding data. (B) Comparison of canine and human 5-HT_{2B} binding data. (C) Comparison of canine 5-HT_{2A} and 5-HT_{2B} binding data. (D) Comparison of human 5-HT_{2A} and 5-HT_{2B} binding data. Correlation coefficient (r^2) and P values are given.

known to display significant selectivity for human 5-HT_{2B} receptor vs. human 5-HT_{2A} receptor (α-Me-5-HT, BW-723C86, SB-2014741, and yohimbine) were found to display a similar selectivity profile on the canine receptor subtypes (Table 2).

3.3. Functional characterization of recombinant canine 5-HT_{2A} and 5-HT_{2B} receptors

To investigate the in vitro functional properties of the canine 5-HT_{2A} and canine 5-HT_{2B} receptors, we used the FLIPR that integrates drug addition and Ca^{2+} fluorescence measurements, allowing rapid detection of Ca^{2+} following receptor activation. Human 5-HT_{2A} and 5-HT_{2B} receptors were also included in this study for comparison.

5-HT stimulated Ca^{2+} response in cells transfected with canine 5-HT_{2A} (HEK-293), human 5-HT_{2A} (NIH3T3), c5-HT_{2B} (HEK-293), or h5-HT_{2B} (CHO) (Fig. 5A and B). No signal to 5-HT was obtained from non-transfected HEK-293, CHO, or NIH3T3 cells that underwent an identical assay protocol (data not shown). NIH3T3 and CHO have been previously shown to expressed 5-HT_{2A} and 5-HT_{1B} receptor, respectively (Giles et al., 1996; Saucier and Albert, 1997). However, the expression level of endogenous receptors in these cell lines is sufficiently low so as not to interfere with the study of recombinant receptors whose expression levels are generally significantly higher.

Higher fluorescence intensities were observed for both canine and human 5-HT_{2A} compared to the canine and human 5-HT_{2B} receptors. A slightly higher fluorescence intensity peak was observed for the human 5-HT_{2A} compared to the canine 5-HT_{2A} (15 800 vs. 13 600 peak fluorescence intensity; Fig. 5A). A weaker response was observed for the canine 5-HT_{2B} receptor (peak fluorescence intensity of 3500) compared to the human 5-HT_{2B} receptor (peak fluorescence intensity of 5000; Fig. 5B). Saturation binding analysis using [^{3}H]5-HT was performed on membranes prepared from HEK-293 cells transfected with the canine 5-HT_{2B} and demonstrated that the expression level (B_{max} = 10.9 ± 0. 30 pmol/mg protein) was lower compared to the expression level obtained in membranes prepared from CHO cells expressing human 5-HT_{2B} (B_{max} = 35.67 ± 7.12 pmol/mg protein).

To further address the difference in peak fluorescence intensity between the human and canine 5-HT_{2B} receptor, the 5-HT stimulated Ca^{2+} response experiment was repeated but using the same cell line background (HEK-293) for both human and canine 5-$HT_{2A/2B}$ receptor (canine 5-HT_{2A}/HEK-293 B_{max} = 6.40 ± 0.90 pmol/mg protein; human 5-HT_{2A}/HEK-293 B_{max} = 9.00 ± 0.40 pmol/mg protein; canine

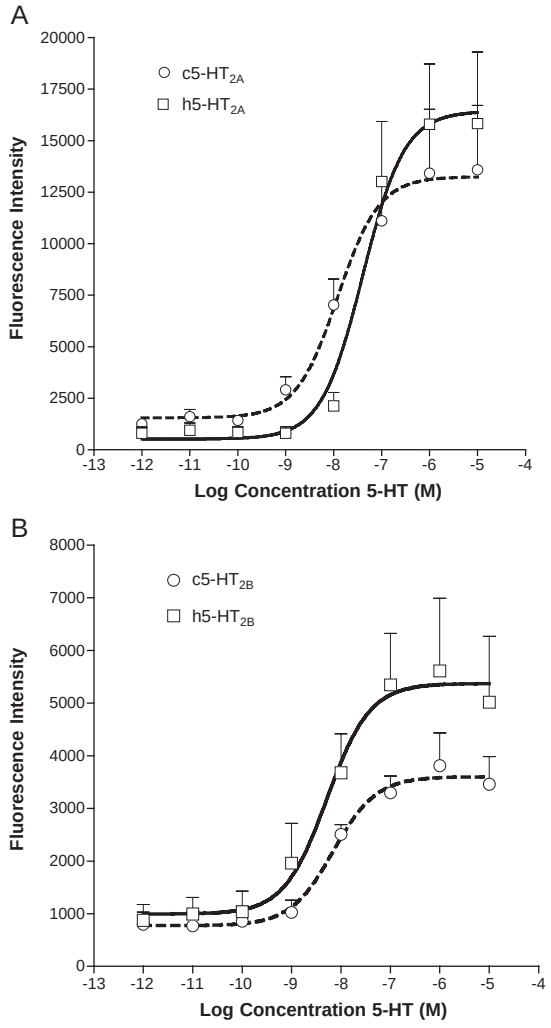

Fig. 5. 5-HT stimulation of cells expressing canine or human 5-HT_{2A} receptors (A), or canine or human 5-HT_{2B} receptors (B). Each data point depicting the average fluorescence value was performed in triplicate (mean ± S.E.M.).

5-HT_{2B}/HEK-293 B_{max} = 10.9 ± 0.30 pmol/mg protein; human 5-HT_{2B}/HEK B_{max} = 8.70 ± 0.30 pmol/mg protein). A significantly higher fluorescence intensity peak after 5-HT stimulation was observed for the human 5-HT_{2B} receptor compared to the canine 5-HT_{2B} receptor, whereas the human and canine 5-HT_{2A} were within the same range (data not shown).

Table 4
Antagonist potency (pK_B) at cloned canine and human 5-HT_{2A} or 5-HT_{2B} receptors

Compound	c5-HT_{2A} HEK-293	h5-HT_{2A} NIH3T3	c5-HT_{2B} HEK-293	h5-HT_{2B} CHO
Ritanserin	8.15 ± 0.19	7.98 ± 0.22	8.00 ± 0.41	8.23 ± 0.22
Risperidone	9.68 ± 0.11	9.13 ± 0.22	8.40 ± 0.97	7.25 ± 0.14
MDL-100907	9.38 ± 0.06	8.88 ± 0.03	<5	<5
Eplivanserin	9.60 ± 0.49	9.43 ± 0.03	<5	<5
SB-204741	<5	<5	7.35 ± 0.45	7.35 ± 0.76
Mesulergine	7.60 ± 0.10	7.23 ± 0.13	9.30 ± 0.45	8.63 ± 0.07
Yohimbine	<5	<5	8.80 ± 0.43	7.35 ± 0.45
Methiothepin	8.33 ± 0.09	7.80 ± 0.10	8.07 ± 0.77	8.28 ± 0.03
Ketanserin	8.90 ± 0.13	8.53 ± 0.48	5.80 ± 0.40	<5
Olanzapine	7.27 ± 0.15	6.93 ± 0.03	6.60 ± 0.13	7.43 ± 0.19

Data are the mean±S.E.M of three to six experiments.

The activity of various other 5-HT_2 receptor agonists, exhibiting a range of potencies and relative efficacies, is summarized in Table 3. The affinities of the antagonists are summarized in Table 4. The rank order of potency and antagonist affinities was generally consistent with the established pharmacology.

For the 5-HT_{2A} receptor, several compounds were found to display higher potency on the canine receptor compared to human 5-HT_{2A} (*m*-CPP, SCH-23390, TFMPP, BW-723C86, and 5-CT). Similarly most of these compounds had higher relative efficacies on the canine 5-HT_{2A} receptor compared to the human 5-HT_{2A} receptor. In general, all the antagonists had similar affinities for the human and canine 5-HT_{2A} receptors except methiothepin, which displays a 11-fold higher affinity for the canine receptor.

For the 5-HT_{2B} receptor, several agonists were found to display lower potency on the canine 5-HT_{2B} receptor compared to the human 5-HT_{2B} receptor. These compounds had lower relative efficacies on the canine 5-HT_{2B} receptor compared to the human 5-HT_{BA} receptor. For *m*-CPP and TFMPP, no agonistic response was observed on canine 5-HT_{2B}.

None of the compounds (10 μM) tested elicited a response in non-transfected cells (HEK-293, NIH3T3, or

Table 3
Agonist potency and relative efficacy at cloned canine and human 5-HT_{2A} or 5-HT_{2B} receptors

Compound	c5-HT_{2A} HEK-293 pEC_{50}	Relative efficacy	h5-HT_{2A} NIH3T3 pEC_{50}	Relative efficacy	c5-HT_{2B} HEK-293 pEC_{50}	Relative efficacy	h5-HT_{2B} CHO pEC_{50}	Relative efficacy
5-HT	7.70 ± 0.46	1	7.38 ± 0.46	1	8.36 ± 0.19	1	8.73 ± 0.23	1
DOI	8.98 ± 0.09	0.89 ± 0.02	8.48 ± 0.09	0.65 ± 0.04	8.10 ± 0.20	0.75 ± 0.03	8.75 ± 0.19	0.96 ± 0.02
m-CPP	7.57 ± 0.11	0.71 ± 0.04	6.70 ± 0.05	0.20 ± 0.02	IA		8.35 ± 0.15	0.55 ± 0.06
SCH-23390	6.68 ± 0.10	0.61 ± 0.03	7.58 ± 0.22	0.17 ± 0.02	5.88 ± 0.87	0.37 ± 0.05	7.10 ± 0.16	0.66 ± 0.05
α-Me-5-HT	8.20 ± 0.60	1.06 ± 0.05	7.58 ± 0.13	0.91 ± 0.02	8.80 ± 0.36	0.77 ± 0.01	8.75 ± 0.17	0.84 ± 0.01
BW-723C86	7.48 ± 0.13	0.89 ± 0.03	6.60 ± 0.07	0.56 ± 0.03	8.14 ± 0.14	0.93 ± 0.03	9.03 ± 0.06	0.98 ± 0.01
TFMPP	7.37 ± 0.13	0.72 ± 0.04	6.63 ± 0.28	0.17 ± 0.01	IA		7.90 ± 0.21	0.49 ± 0.04
5-CT	7.70 ± 0.13	0.94 ± 0.08	6.80 ± 0.09	0.70 ± 0.13	8.00 ± 0.15	1.19 ± 0.05	8.85 ± 0.18	0.95 ± 0.01

pEC_{50} values are the mean±S.E.M of three to six independent experiments. Relative efficacy values are the corresponding fraction of the response elicited by the compounds compared to 10 μM 5-HT. IA: inactive.

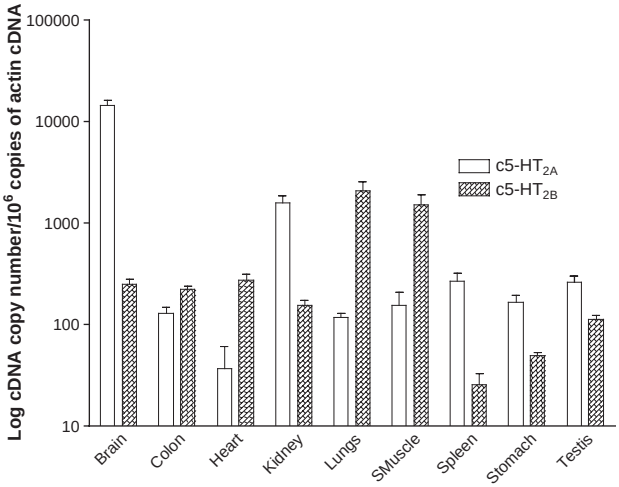

Fig. 6. Quantitative RT-PCR detection of canine 5-HT_{2A} and canine 5-HT_{2B} receptor mRNA in various canine tissues. Quantitative PCR analyses were performed to measure the abundance of canine 5-HT_{2A} and c5-HT_{2B} receptor mRNA from nine different canine tissues, respectively. In parallel, PCR measurement for β-actin gene expression in different tissues served as internal controls. The canine 5-HT_{2A} and canine 5-HT_{2B} cDNA expression levels were normalized to the actin expression levels for each tissue. Error bars indicate standard deviation.

CHO). In addition, none of the antagonists tested increased Ca^{2+} in any of the transfected cell types (data not shown).

3.4. Expression of 5-HT_{2A} and 5-HT_{2B} receptor mRNAs in canine tissue

Based on recombinant canine 5-HT_{2A} and 5-HT_{2B} specific receptor sequence, primers were designed and used on cDNA templates prepared from several canine tissues. The quantitative expression profiles of canine 5-HT_{2A} and canine 5-HT_{2B} are shown in Fig. 6. The canine 5-HT_{2A} receptor was mainly detected in the brain and at lower levels in peripheral tissues, whereas canine 5-HT_{2B} was detected mainly in lungs and smooth muscles and at a lower level in the brain. The following order of expression levels was observed for canine 5-HT_{2A} receptor mRNA: brain ⩾ kidney > spleen > testis > stomach > smooth muscle > colon > lungs > heart and for canine 5-HT_{2B} receptor mRNA: lungs > smooth muscle > heart > brain > colon > kidney > testis > stomach > spleen.

4. Discussion

In the present study, the cloning and molecular characterizations of the canine 5-HT_{2A} and 5-HT_{2B} receptors are described.

We had independently cloned the canine 5-HT_{2A} cDNA before the canine genome sequence was released. A recent search of Genbank identified a canine 5-HT_{2A} cDNA sequence (Genbank accession no. NM_001005869; Masuda et al., 2004), which is identical to our sequence. The canine 5-HT_{2A} receptor is a protein of 470 amino acids with seven hydrophobic transmembrane domains (Fig. 1) and shares 93% amino acid homology with the human 5-HT_{2A} receptor. Similar to its human homolog, the canine 5-HT_{2A} receptors contains conserved amino acid residue (Ser^{242}) in the fifth transmembrane domain. An amino acid variation in that position (242) has been demonstrated to account for pharmacological differences between the rat (Ala^{242}) and human (Ser^{242}) 5-HT_{2A} receptors (Roth et al., 1998). The rat receptor displays higher affinity for the ergoline-based compounds compared to the human receptor. In the present radioligand competition binding study, a very tight correlation between the canine and human 5-HT_{2A} receptor was found for the 19 serotoninergic ligands investigated (Table 2, Fig. 4A). The largest difference in affinity was observed for ketanserin and eplivanserin (fivefold higher affinity for human vs. canine 5-HT_{2A} receptor). In general, the canine 5-HT_{2A} receptor behaves similarly to the human 5-HT_{2A} receptor in the Ca^{2+} mobilization assay. All the compounds found to behave as agonists, partial agonists, or antagonists on the human 5-HT_{2A} receptor were also found to be agonists, partial agonists, or antagonists, respectively, on the canine 5-HT_{2A} receptor. 5-HT elicited a similar Ca^{2+} response (fluorescence intensity peak) on human and canine 5-HT_{2A} receptors (Fig. 5A). In general, our pIC_{50} and pK_B values for the human 5-HT_{2A} receptor were in good agreement with the values reported in the literature with various cell lines (Jerman et al., 2001; Porter et al., 1999), except for methiothepin (pK_B in this study 7.80 vs. 9.03 as reported by Jerman et al., 2001). In the present study, we have observed discrepancies between the binding pK_i and functional pIC_{50} or pK_B values for both human and canine 5-HT_2 receptors. However, these two sets of data cannot be directly compared since we used antagonist radioligands for competition binding. Antagonists usually do not show a marked difference in binding affinity between coupled and uncoupled state of the 5-HT_2 receptor subtypes. Noteworthy, we also used different cell lines for binding and functional assays (COS-7 vs. HEK-293, respectively) for reasons previously discussed in the literature (Chen et al., 2003). Another possible reason that ligands display different binding potencies and functional pIC_{50} or pK_B values could be that the binding assay is an equilibrium assay while Ca^{2+} mobilization is closer to a kinetic assay. For several agonists or partial agonists (i.e., *m*-CPP, SCH-23390, BW-723C86, TFMPP, and 5-CT), relatively higher potencies (five- to ninefold) and relative efficacies were observed on the canine 5-HT_{2A} receptor compared to the human 5-HT_{2A} receptor. However, for the natural ligand (5-HT), only a twofold higher potency on the canine 5-HT_{2A} was observed. Antagonistic affinities for the canine 5-HT_{2A} vs. the human 5-HT_{2A} receptors were within a threefold ratio excepted for methiothepin (for which a rather low pK_B was obtained for the human 5-HT_{2A} in this study compared to the literature; see above).

The canine 5-HT_{2B} receptor is a protein of 434 amino acids with seven hydrophobic transmembrane domains (Fig.

2). The canine and human 5-HT_{2B} receptors are also highly conserved (87% homology) with the exception of the carboxyl termini where the canine protein is missing the last 62 amino acids of the human 5-HT_{2B} receptor. DNA sequence comparison between the human and canine 5-HT_{2B} receptors indicates that there is a sequence in the 3′ end of canine 5-HT_{2B} cDNA that is highly homologous to the sequence coding for the C-terminal 62 amino acids compared to the human 5-HT_{2B} receptor. However, in canine 5-HT_{2A} cDNA, at position corresponding to the 1254 position of the human 5-HT_{2B} coding region, there is a 5-nucleotide deletion (Fig. 7A), which results in a reading frame shift in the canine 5-HT_{2B} mRNA. Since this reading frame shift in canine 5-HT_{2B} cDNA also causes an early termination of the translation, the canine 5-HT_{2B} receptor protein is significantly shorter at the C-terminus.

We searched DNA sequences in other species and found that 5-HT_{2B}-like DNA sequences encoding very similar C-terminus to that of the human 5-HT_{2B} were identified from frog, puffer fish, zebra fish, and chicken, in addition to mouse and rat (Fig. 7B). These results suggest that 5-HT_{2B} gene is very conserved among different species. The fact that canine 5-HT_{2B} has a C-terminal truncation indicates that canine 5-HT_{2B} is a unique or different member in the 5-HT_{2B} genes from different species. However, whether the truncation in the canine 5-HT_{2B} receptor has any physiological relevance remains to be studied.

In the present radioligand competition binding study, a very tight correlation between the canine and human 5-HT_{2B} receptors was found for the 19 serotoninergic ligands investigated (Table 2, Fig. 4B). The largest differences in affinity were observed for BW-723C86 and olanzapine (threefold higher affinity for human vs. canine 5-HT_{2A} receptor). In contrast, substantial differences in Ca^{2+} mobilization were observed. 5-HT elicited a stronger Ca^{2+} response on the human 5-HT_{2B} receptor compared to the canine 5-HT_{2B} receptor (Fig. 5B). This observation was further confirmed in an experiment where the canine 5-HT_{2B} and human 5-HT_{2B} cDNA were transiently transfected in parallel in HEK-293 cells. In addition, two structurally related partial agonists on the human 5-HT_{2B} receptor (*m*-CPP and TFMPP) did not elicit any functional response on the canine 5-HT_{2B} receptor up to 10 μM. The C-terminal region of GPCR is well known to be important for signal transduction. Hazelwood and Sanders-Bush (2004) recently reported that a mutation in the C-terminal tail ($His^{452}Tyr$) destabilizes the signaling of human 5-HT_{2A} receptor and results in lower ligand-stimulated responses. Therefore, given that there is a truncation in the C-terminus region of the canine 5-HT_{2B}, the weaker Ca^{2+} response observed in cells expressing the canine 5-HT_{2B} receptor was not unexpected. Noteworthy, as previously reported by Porter et al. (1999), the human h5-HT_{2B} receptor consistently elicits a smaller Ca^{2+} signal. It has been suggested that the 5-HT_{2B} receptor may couple less efficiently to PLC, and that other signal transduction cascades may be physiologically relevant. The present data (i.e., deletion of 62 amino acids in the C-terminal region and weaker Ca^{2+} mobilization response) reinforce this hypothesis. Manivet et al. (2000) have shown that in transfected cells expressing a partial C-

A

```
                *1180     *1190     *1200     *1210     *1220     *1230     *1240     *1250
     Human: TTTGGCCGATATATCACCTGCAATTACCGGGCCACAAAGTCAGTAAAAACTCTCAGAAAACGCTCCAGTAAGATCTACTT
Consensus: TTTGGCCGATATAT ACCTGCAATTACC GGCCA  AA TCAGTAAAAACT TCAGAAAA GCTCCAG AA A CTACTT
    Canine: TTTGGCCGATATATTACCTGCAATTACCAGGCCATGAAATCAGTAAAAACTGTCAGAAAATGCTCCAGCAATAACTACTT
              ^1170     ^1180     ^1190     ^1200     ^1210     ^1220     ^1230     ^1240

                *1260     *1270     *1280     *1290     *1300     *1310     *1320     *1330
     Human: CCGGAATCCAATGGCAGAGAACTCTAAGTTTTTCAAGAAACATGGAATTCGAAATGGGATTAACCCTGCCATGTACCAGA
Consensus: CCG      AATGGCAGAGAACTC AAGTTTTTCA GAAAC TGGAAT  GAAATGG ATTAA CCTGCCA GTACCAGA
    Canine: CCGA-----AATGGCAGAGAACTCAAAGTTTTTCATGAAACGTGGAATGTGAAATGGTATTAATCCTGCCACGTACCAGA
              ^1250     ^1260     ^1270     ^1280     ^1290     ^1300     ^1310     ^1320
                *1340     *1350     *1360     *1370     *1380     *1390     *1400     *1410
     Human: GTCCAATGAGGCTCCGAAGTTCAACCATTCAGTCTTCATCAATCATTCTACTAGATACGCTTCTCCTCACTGAAAATGAA
Consensus: G CCAATGAGGCTC G AGTTCAACCATTCA  CTTC TCAATCATTCTACTAGATAC CTTCTC TCAC GAAAATGAA
    Canine: GCCCAATGAGGCTCTGCAGTTCAACCATTCACGCTTCCTCAATCATTCTACTAGATACACTTCTCATCACAGAAAATGAA
            ^1330     ^1340     ^1350     ^1360     ^1370     ^1380     ^1390     ^1400

                *1420     *1430     *1440
     Human: GGTGACAAAACTGAAGAGCAAGTTAGTTATGTATAG
Consensus: GGTGACAAAACTGAAGAGCAAGT AGTTATGTATAG
    Canine: GGTGACAAAACTGAAGAGCAAGTCAGTTATGTATAG
            ^1410     *1420     *1430
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B

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          *410      *420      *430      *440      *450      *460      *470      *480    Genbank No
     Human: KRSSKIYFRNPMAENSKFFKKHGIRNGINPAMYQSPMRLRSSTIQSSSIILLDTLLLTENEGDKTEEQVSYV      AY136751
     Mouse: KFSSTLCFGNSMVENSKFFTKHGIRNGINPAMYQSPMRLRSSTIQSSSIILLDT-LLTENDGDKAEEQVSYI      CAA78824
      Rat: KCSSTLYFGNSMVENSKFFTKHGIRNGINPAMYQSPVRLRSSTIQSSSIILLNT-FLTENDGDKVEDQVSYI      NM_017250
     Frog: NCSSRISFRNSMAENSKLIMKHGMKNGINPVMYQSPLRLCNAQLESSA-ILLDTLLLTENEAGKTEEQASYV      CAD71264
  Chicken: KCSSRISFRNSVTENSKLFVMHGMRNGINPIMYQSPMRLRSSPIQASSAILLDTLLLTENEADKTEEQVSYV      NW06421
Puffer fish: RMLTHISPRSSVAENAKLFTKQEIKN--ETTDYRSPLGCLQPSAQTSTGVVLDKILLTHTENCKQEERVSCV      CAC85912
Zebra fish: RR-SKISFRSSVTENSKRFMKHGMKNGISPVGYQSPIRHRSTQLQTSANIMLDTLLLTDNEDCKPDEHVSHV      BX57193
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Fig. 7. (A) Nucleotide sequence comparison between the human and canine 5-HT_{2B} cDNA. The 5-nucleotide deletion in canine 5-HT_{2A} cDNA is shown. The numbers indicate the nucleotide position in cDNA starting with the translation starting codon (ATG). The putative translation stop codons for the human and canine 5-HT_{2B} receptors are underlined. (B) C-terminal sequence comparison among human, mouse, rat, chicken, frog, puffer fish, and zebra fish 5-HT_{2B} receptor proteins. The numbers indicate the amino acid position corresponding to the human 5-HT_{2B} receptor.

terminus truncated form of the human 5-HT_{2B} receptor (K403X), DOI-dependant IP_3 coupling was retained. Interestingly, a heterozygous mutation R393X in the human 5-HT_{2B} receptor was identified in one patient diagnosed with pulmonary hypertension after intake of dexfenfluramine (Blanpain et al., 2003). More recently, Deraet et al. (2005) have shown that the lack of C-terminus in human 5-HT_{2B} receptor (R393X) can generate a switch of coupling to $G_{\alpha 13}$, a reduced NO synthase activation, and an increase in cell proliferation.

The tissue distribution of the canine 5-HT_{2A} and canine 5-HT_{2B} receptor mRNAs (Fig. 6) was similar to that of the human 5-HT_{2A} and human 5-HT_{2B} receptor mRNAs, respectively (Leysen, 2004). The highest expression level of canine 5-HT_{2A} mRNA was observed in brain tissue, whereas the highest expression levels of canine 5-HT_{2B} were in the periphery. However, significant levels of canine 5-HT_{2B} receptor mRNA were detected in the brain. Study of the function of the 5-HT_{2B} receptor in the brain has not attracted much attention, most likely because of the early reports where 5-HT_{2B} mRNA could not be detected in rat brain. However, several studies, including this one, have clearly demonstrated the presence of a significant level of 5-HT_{2B} mRNA in rat, human, and canine brains (Bonaventure et al., 2002; Duxon et al., 1997; Kursar et al., 1994).

In conclusion, in the present study, we have identified and characterized canine 5-HT_{2A} and canine 5-HT_{2B} receptors. The canine 5-HT_{2A} receptor is pharmacologically close to the human homolog, whereas the canine 5-HT_{2B} is showing significant differences in the C-terminal region, translating into weaker functional response compared to human 5-HT_{2B} receptor. The availability of the full sequence and pharmacology of the canine 5-HT_{2A} and canine 5-HT_{2B} receptors provides useful information for the interpretation of previous and future pharmacological studies of 5-$HT_{2A/2B}$ ligands in canine.

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