

Short Sequence-Paper

Species differences in 5-HT_{2A} receptors: cloned pig and rhesus monkey 5-HT_{2A} receptors reveal conserved transmembrane homology to the human rather than rat sequenceMichael P. Johnson^{b,*}, Melvyn Baez^a, J. David Kursar^a, David L. Nelson^a^a Lilly Research Laboratories, CNS Research, Lilly Corporate Center, Indianapolis, IN 26285, USA^b Marion Merrell Dow Research Institute, Cerebrovascular Biology, 2110 E. Galbraith Road, Cincinnati, OH 45215, USA

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Abstract

Pig and rhesus monkey 5-HT_{2A} receptor cDNA clones were isolated. The pig and rhesus monkey clones encode proteins that share a 94% and 95% homology, respectively, with the rat 5-HT_{2A} receptor, and a 97% and > 99% homology, respectively, with the human 5-HT_{2A} receptor. Within the transmembrane regions of the pig and monkey receptors, the deduced amino acid shows only three differences compared to that of the rat and are identical to the human 5-HT_{2A} receptor clone.

Keywords: 5-HT_{2A} receptor; cDNA sequence; (Pig); (Rhesus monkey)

The 5-HT_{2A} receptor was one of the first three 5-HT receptors to be cloned. The amino acid sequence of the rat [1–3], hamster [4] mouse [5] and more recently the human [5–9] 5-HT_{2A} receptors have been reported. The homology of these 5-HT_{2A} receptor sequences between the different species is quite high with only three amino acid changes found within the transmembrane (TM) regions, where ligand binding is thought to occur (for a review see [10]). Despite the high degree of homology, there are well documented differences in the pharmacological profile of the rat and human 5-HT_{2A} receptors [11–13].

Previously it was found that mesulergine and methysergide (ergolines that act as antagonists at the 5-HT_{2A} receptor) had different affinities for the rat 5-HT_{2A} receptor than for the pig or human 5-HT_{2A} receptors [11–13]. Recent work has identified a very specific structure-activity relationship difference among the 5-HT_{2A} receptors of various species [12,13]. These studies have indicated that N(1) alkyl substitution of indole-containing ligands either increases or does not effect the affinity for the rat 5-HT_{2A} receptor but significantly decreases affinity for the pig, squirrel monkey and human 5-HT_{2A} receptors.

The present study was undertaken for two reasons. First, to determine whether the same pharmacological profile for 5-HT_{2A} receptor ligands would be seen with rhesus monkey frontal cortex as has been previously reported for the pig, squirrel monkey and human 5-HT_{2A} receptors. Secondly, cDNA clones encoding the 5-HT_{2A} receptor in pig and rhesus monkey were isolated and sequenced to determine and compare the amino acid sequence homologies of the 5-HT_{2A} receptor in the rat, human, pig and rhesus monkey.

Materials were obtained from the following sources. [³H]Ketanserin and [α -³²P]dCTP was obtained from New England Nuclear (Boston, MA) at a specific activity of 60 and 3000 Ci/mmol, respectively. Ketanserin HCl, spiperone HCl, ergonovine maleate, mesulergine HCl, metergoline and LY53857 maleate were purchased from Research Biochemicals Inc. (Natick, MA). All other compounds were synthesized at Lilly Research Laboratories (Indianapolis, IN). A cDNA encoding the rat 5-HT_{2A} receptor [2] was kindly provided by Dr. Lei Yu (Indiana University-Purdue University of Indianapolis, Indianapolis, IN).

Affinity for the rhesus monkey 5-HT_{2A} receptor was examined as described by Nelson et al. [12] with only minor modifications. Specifically, rhesus monkey brains were obtained from the Texas Primate Center (Alice, TX) and the anterior one third utilized in these studies. The

* Corresponding author. Fax: +1 (513) 9486439.

tissue was prepared as described [12] and then resuspended after the final wash in 50 mM Tris-HCl (pH 7.6) at 25 mg wet wt./ml and frozen at -70°C until used. Assays were automated using a Biomek 1000 (Beckman Instruments, Fullerton, CA) in a final volume of 250 μl . Radioligand, 25 μl of 1 nM (final concentrations) in displacement experiments or 25 μl of 0.2 to 10 nM in saturation experiments, was added to buffer and varying concentrations of drugs (dissolved in H_2O) such that the final buffer consisted of 50 mM Tris-HCl (pH 7.6), with 100 nM prazosin. Non-specific binding was defined as that displaceable with 3 μM spiperone. Incubations were commenced with the addition of 100 μl tissue homogenate and allowed to equilibrate at 37°C for 30 min before filtering through GF/B filters presoaked in 0.5% polyethylenimine. Saturation and displacement results were analyzed as previously described [12,13].

Standard cDNA cloning procedures were utilized essentially as described in the methods of Kursar et al. [14] with minor modifications. Pig and rhesus monkey cDNA libraries were obtained from Clontech (San Francisco, CA). These libraries had undergone 1 round of amplification. The libraries were plated at a density of $5 \cdot 10^5$ pfu/plate on 150 mm dishes using standard procedures. The nitrocellulose filters obtained from plaque lifts were prehybridized at 45°C overnight in 30% formamide, $5 \times \text{SSPE}$, $5 \times \text{Denhardt's}$, 0.1% SDS, and 100 $\mu\text{g/ml}$ denatured salmon sperm DNA. A radiolabelled probe was synthesized by PCR labeling as previously described [15] using the cDNA encoding the rat 5-HT_{2A} receptor as a template. Hybridization was carried out at 45°C for 48 h and then the filters washed three times for 10 min at 45°C in $2 \times \text{SSC}/0.1\%$ SDS and then washed three times for 30 min at 45°C in $0.1 \times \text{SSC}/0.1\%$ SDS. Several clones were isolated from each library and the clones bearing the largest cDNA inserts were further analyzed. It was found that the cDNA clones contained an internal *EcoRI* site (the enzyme used in the library construction). Therefore, the resulting fragments were each isolated and subcloned into

pSport vector (Gibco BRL, Gaithersburg, MD) for further analysis. The cDNA clones were sequenced using the ABI 373 Automated Sequencer (ABT, Foster City, CA) and the procedures provided by the vender. The University of Wisconsin GCG software was utilized for all sequencing analysis.

Saturation experiments in homogenates from rhesus monkey frontal cortex indicated a K_d of 0.66 ± 0.04 nM and a B_{max} of 299 ± 49 fmol/mg protein ($n = 3$) for [^3H]ketanserin. In displacement experiments (Table 1) ketanserin, spiperone and metergoline were all found to have similar affinity for the rat, human, pig squirrel monkey and rhesus monkey 5-HT_{2A} receptor [12,13]. The N(1) substituted ergolines, mesulergine and LY53857, have a lower affinity for the rhesus monkey 5-HT_{2A} receptor than the rat receptor (Table 1). In contrast, the N(1) unsubstituted ergolines, ergonovine and LY86057, have a higher affinity for the rhesus monkey 5-HT_{2A} receptor than the rat receptor. All of these ergolines were found to have affinities for the rhesus monkey that were very similar to those found for the squirrel monkey, pig and human receptors.

The nucleotide sequence of the rhesus monkey clone isolated from the cDNA library appeared to contain a deletion of one nucleotide in the region encoding the carboxy terminus. A series of adenosines found at positions 1283 to 1289 contained one less base than seen in any other 5-HT_{2A} receptors. It was hypothesized that the apparent deletion of a single base pair within this region (1283–1290) was due to an error in the synthesis of cDNA with reverse transcriptase. To test this hypothesis, a 400 base pair DNA fragment was amplified from rhesus monkey genomic DNA (Clontech, San Francisco, CA) by PCR using standard procedures and sequenced. The resulting PCR product confirmed a string of 7 adenosines consistent with the other 5-HT_{2A} receptor sequences (as opposed to 6 found in the λ phage clone). Therefore, it is presumed that the isolated cDNA clone sequence contained an unnatural deletion and the corrected sequence (as given in Fig. 2) was used for all subsequent analysis.

Table 1

Affinity for [^3H]ketanserin-labeled rhesus monkey 5-HT_{2A} receptors in frontal cortex homogenates

Drug	N(1) subst.	IC ₅₀ (nM)				
		Rhesus monkey ^a	Rat ^b	Squirrel monkey ^b	Pig ^b	Human ^b
Ketanserin		3.35 ± 0.09	2.49		4.28	1.70
Spiperone		2.16 ± 0.26	$0.92^c (\pm 0.05)$			
Metergoline	-CH ₃	1.19 ± 0.04	0.76	0.82	0.37	1.2
Ergonovine	-H	3.22 ± 0.99	46.4	3.4	3.8	3.1
Mesulergine	-CH(CH ₃) ₂	45.9 ± 10.3	8.2	44.9	54.2	48.5
LY86057	-H	4.20 ± 0.30	55.9	3.2	4.6	3.3
LY53857	-CH(CH ₃) ₂	57.5 ± 3.8	7.2	33.1	40.0	30.6

IC₅₀ values were determined by displacement of 1.0 nM [^3H]ketanserin as described previously [12]. The IC₅₀ values for displacement from rat, squirrel monkey, pig and human [^3H]ketanserin-labelled 5-HT_{2A} receptors were included for comparison [12].

^a Mean \pm S.E. (n).

^b Taken from [12].

^c Nelson, D.L., Wainscott, B.D. and Lucaites, V.L., unpublished results.

The cDNA sequence encoding the pig and rhesus monkey 5-HT_{2A} receptors are given in Figs. 1 and 2, respectively. At the nucleotide level the pig 5-HT_{2A} receptor has 85% homology to the rat and 91% homology to the human analogue of this receptor. Similarly the rhesus monkey 5-HT_{2A} receptor has 87% homology to the rat and 98% homology to the human analogue of this receptor. A multiple sequence alignment of the deduced amino acids indicates a high homology to the rat 5-HT_{2A} receptor (94% and 97%, for the pig and rhesus monkey, respectively) and an even higher homology to the human 5-HT_{2A} receptor (95% and > 99% for the pig and rhesus monkey, respectively). Within the TM regions, the pig and rhesus monkey sequences are identical to the human amino acid sequence

but differ by three amino acids from that of the rat sequence (Fig. 3).

Previous work using mutagenesis of the human and rat 5-HT_{2A} receptors has suggested that the changes in structure–activity relationship seen when examining different species are due to a single amino acid difference between the rat and human 5-HT_{2A} receptor. Kao and co-workers [16] found that by mutating Ser-242 in the TM V region of the human 5-HT_{2A} receptor to Ala-242 there was an increase in the affinity for [³H]mesulergine while the affinity for [³H]ketanserin was unaffected. Specifically, [³H]mesulergine was found to have an affinity for the mutated 5-HT_{2A} receptor that was very similar to the rat but different than the wild-type human 5-HT_{2A} receptor.

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      0      20      40      60
TCCGACATGGATGTTCTCTGTGAAGAAACACTTCTTTGAGCTCACCTACGAACTCCCTCATGCAATTAACGATGACAC
1  M D V L C E E N T S L S S P T N S F M Q L N D D T 27
      80     100     120     140
AAGGCTCTACCAATAACGACTTTAACTCGGAGAAGCTAACTTACAGCAGCATTTAACTGGACCGTGGACTCAGAAAATC
27 R L Y H N D F N S G E A N T S D A F N W T V D S E N R 53
      160     180     200     220
GAACCAACCTTTCTCTGTGAGGGCTGTCTCTCACCACCTGCTTCTCTTCTCATCTCCAGGAAAAAACTGGTCTGCC
54 T N L S C E G C L S P P C F S L L H L Q E K N W S A 80
      240     260     280     300
TTGTTGACAGCTGTAGTGATTATCTCTGACCATTTGCTGGAACATACTCGTCATCATGGCAGTGTCCCTAGAGAAAAGCT
81 L L T A V V I I L T I A G N I L V I M A V S L E K K L 107
      320     340     360     380
CGAGAATGCCACCAACTATTTCTGTATGTCACTTGCCATAGCGGATATGCTGCTGGGTTTCTTGTCTATGCCTGTGTCCA
107 Q N A T N Y F L M S L A I A D M L L G F L V M P V S M 133
      400     420     440     460
TGTTAACCATTTCTCTATGGGTACCGTGGCTCTGCCCCAGCAAGCTCTGCGCCGCTCTGGATTATCTGGATGTGCTCTTC
134 L T I L Y G Y R W P L P S K L C A V W I Y L D V L F 160
      480     500     520     540
TCCACGGCCCTCCATCATGCACTCTGTGCCATCTCGCTGGACCGCTATGTTGCCATCCAGAACCCATCCATCACAGGCG
161 S T A S I M H L C A I S L D R Y V A I Q N P I H H R R 187
      560     580     600     620
ATTCAACTCTAGAACTAAGGCATTTCTGAAAATAATGTCTGTTGGACTATATCAGTAGGTATCTCCATGCCAATCCCTG
187 F N S R T K A F L K I I A V W T I S V G I S M P I P V 213
      640     660     680     700
TCTTTGGGCTCCAGGATGATTCCAAGGTCTTCAAGGAAGGAGCTGCTTACTTGGCGATGATAATTTGTCTGTGATTGGC
214 F G L Q D D S K V F K E G S C L L A D D N F V L I G 240
      720     740     760     780
TCTTTTGTGTCATTTTTCATTCCTTAACCATCATGGTGATCACTACTTTCTTAACCATCAAGTCACTCCAGAAAGAGC
241 S F V S F F I P L T I M V I T Y F L T I K S L Q K E A 267
      800     820     840     860
TACTTTGTGTGTGAGTGATCTTTGGACACGGGCCAAGTTAGCTTCTTTTCAGCTTCTTCCCTCAGAGCTCCCTGTCTTCGG
267 T L C V S D L G T R A K L A S F S F L P Q S S L S S E 293
      880     900     920     940
AAAAGCTCTTCCAGCGTCCATCCACAGGAGCCAGGCTCTACGGCAGGAGCTATGCAGTCCATCAGCAACGAGCAG
294 K L F Q R S I H R E P G S Y G R R T M Q S I S N E Q 320
      960     980     1000    1020
AAGCGTGCAAGGTGCTGGGCATAGTCTTCTTCTGTCTGTTGGTGATGTTGGTCCCTTCTTCATCACCAACATCATGGC
321 K A C K V L G I V F F L F V V M W C P F F I T N I M A 347
      1040    1060    1080    1100
CGTCATCTGCAAGAGTCTCTGCAACGAGGATGTCTCGAGCCCTGCTCAACGTGTTCTGTTGGATCGGTTACCTCTCTCT
347 V I C K E S C N E D V I G A L L N V F V W I G Y L S S 373
      1120    1140    1160    1180
CAGCGTCAACCCGCTAGTGTACACACTGTCTAATAAGACCTACAGGTGCGGCTTTTCCAGGTATATTCAGTGTCTAGTAC
374 A V N P L V Y T L F N K T Y R S A F S R Y I Q C Q Y 400
      1200    1220    1240    1260
AAGGAAATAAAAAACCGCTGCTAGTTAATTTAGTGAACACCATACCGGCTTGGCTTACAAGTCTAGTCAACTCAAAAC
401 K E N K K P L Q L I L V N T I P A L A Y K S S Q L Q T 427
      1280    1300    1320    1340
GGGCAAAAAGAGAAATTTCAAAGCAAGCAGACAGGCCACAGAAACGACTGCACTATGGTTGCTTAGGAAAAACAACATT
427 G Q K E N S K Q D D K A T E N D C T M V A L G K Q H S 453
      1360    1380    1400    1420
CAGAAGATGCTCTGAGCAATAGCAACACAGTGAACGAAAGGTTAGCTGTGTATGATGGAGTGGTTGCC
454 E D A P A D N S N T V N E K V S C V * 477

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Fig. 1. The nucleotide sequence and deduced amino acid sequence encoding the pig 5-HT_{2A} receptor. Sequences were isolated from a cDNA λ library using a radiolabelled rat 5-HT_{2A} probe and low stringency hybridization.

Recent work [17] has examined a series of ergolines and tryptamines, that have previously been identified as having species differences in their affinity, at several mutants of position 242 in the rat 5-HT_{2A} receptor. The Ala-242 to Ser-242 mutant, resulted in affinities for the test compounds that were significantly different than seen with the wild-type rat receptor but practically identical to the human 5-HT_{2A} receptor. This clearly indicates that the changes in affinity and therefore the differences in structure-activity relationship found among the 5-HT_{2A} receptors of various species are due to the single amino acid change within TM V.

Pharmacological characterization of the pig [12] and rhesus monkey (Table 1) 5-HT_{2A} receptor have indicated a structure-activity relationship with ergolines and

tryptamines that is very similar to that seen with the human but different from that of the rat 5-HT_{2A} receptor. Since the mutagenesis studies described above clearly implicate an Ala-242 to Ser-242 change between species as a cause for these structure-activity relationship differences, one would predict that the pig and rhesus monkey 5-HT_{2A} receptors would have a Ser and not an Ala at position 242. This was indeed found to be true. Therefore, the present results are consistent with the hypothesis that the Ala/Ser change in the 5-HT_{2A} receptor sequences result in changes in the structure-activity relationship with ergolines and tryptamines.

One of the obvious implications of the present and previous work [11–13,16,17] is that the affinity for the rat 5-HT_{2A} receptor does not serve as a good indicator for

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      0          20          40          60
TGGCTTAGACATGGATATTTCTTGTGAAGAAATACTTCTTTGAGCTCAACTACGAACCTCCTTAATGCAATTAAATGAAG
1  M D I L C E E N T S L S S T T N S L M Q L N E D 27
      80          100         120         140
ACACCAAGGCTCTACAGTAATGACTTTAACTCCGAGAGCTAACACTTCTGATGCATTTAACTGGACAGTCGAGTCGAA
27  T R L Y S N D F N S G E A N T S D A F N W T V E S E 53
      160         180         200         220
AATCGAACCAACCTTTCTCTGTGAAGGGTGCCTCTCACCATCGTGTCTCTCCTTACTTCATCTCCAGGAAAAAATGGTC
54  N R T N L S C E G C L S P S C L S L L H L Q E K N W S 80
      240         260         280         300
TGCTTTATTGACAGCTGTAGTGATTATCTAACCAATTGCTGGAACATACTCGTCATCATGGCAGTGTCCCTAGAGAAAA
81  A L L T A V V I I L T I A G N I L V I M A V S L E K K 107
      320         340         360         380
AGCTGCAGAAATGCCCAACTATTTCTGATGTCACTTGCCATAGCTGATATGCTGCTGGGTTCCTTTGTCATGCCCGTG
107  L Q N A T N Y F L M S L A I A D M L L G F L V M P V 133
      400         420         440         460
TCCATGTTAACCATCTGTATGGGTACCGGTGGCCCTCTGCCAGCAAGCTTTGGCGGGTCTGGATTACCTAGACGTGCT
134  S M L T I L Y G Y R W P L P S K L C A V W I Y L D V L 160
      480         500         520         540
CTTCTCCACGGCTCCATCATGCACCTCTGCGCATCTCGCTGGACCGCTATGTCGCCATCCAGAAATCCCATCCACCACA
161  F S T A S I M H L C A I S L D R Y V A I Q N P I H H S 187
      560         580         600         620
GCCGCTTCAACTCCAGAACTAAGGCATTTCTGAAAATCATTGCTGTTTGGACCATATCAGTAGGTATATCGATGCCAATA
187  R F N S R T K A F L K I I A V W T I S V G I S M P I 213
      640         660         680         700
CCAGTCTTTGGGTACAGGACGATTCGAAGGTCTTTAAGGAGGGGAGTTGCTTACTCGCCGATGATAACTTTGTCTTGAT
214  P V F G L Q D D S K V F K E G S C L L A D D N F V L I 240
      720         740         760         780
CGGCTCTTTTGTGTCATTTTTCATTCCTTGACCATCATGGTGATCACTACTTTCTAACTATCAAGTCACTCCAGAAAG
241  G S F V S F F I P L T I M V I T Y F L T I K S L Q K E 267
      800         820         840         860
AAGTACTTTTGTGTAAAGTATCTCGGCACAGGGCCAAATAGCTTCTTTTCAGCTTCTCCCTCAGAGTTCTTTGTCT
267  A T L C V S D L G T R A K L A S F S F L P Q S S L S 293
      880         900         920         940
TCAGAAAAGCTGTTCCAGCGGTGATCCACAGGACCCAGGGTCTACACAGGCAGAAAGACTATGCAGTCCATCAGCAA
294  S E K L F Q R S I H R D P G S Y T G R R T M Q S I S N 320
      960         980         1000        1020
TGAGCAAAAGGCATGCAAGGTGCTGGGTATCGTCTTCTTCTGTTTGTGGTGATGTGGTGGCTTTCTTCAATCAGCAACA
321  E Q K A C K V L G I V F F L F V V M W C P F F I T N I 347
      1040        1060        1080        1100
TCATGGCCGTCATCTGCAAGAGTCTTGCAATGAGGATGTCATTGGGGCCCTGCTCAATGTGTTTGTGATCGGTTAT
347  M A V I C K E S C N E D V I G A L L N V F V W I G Y 373
      1120        1140        1160        1180
CTCTCTTTCAGCAGTCAACCCACTGGTCTACACACTGTTCACAAAGACTTATAGTTCAGCCTTTTTCAGGTTATATTCAGTG
374  L S S A V N P L V Y T L F N K T Y R S A F S R Y I Q C 400
      1200        1220        1240        1260
TCAGTACAGGAAAAACAAAAACCATTCAGGTAAATTTAGTGAACACTATACCGGCTTTGGCCTACAAAGTCTAGCCAAC
401  Q Y K E N K K P L Q L I L V N T I P A L A Y K S S Q L 427
      1280        1300        1320        1340
TTCAAAATGGGACAAAAAAGAAATTCAAAGCAAGATGCCAAGACAGATAATGACTGCTCAATGGTTGCTCTAGGAAAG
427  Q M G Q K K N S K Q D A K T T D N D C S M V A L G K 453
      1360        1380        1400        1420
CAGCATCTGGAAGATGCTTCTAAAGACAATAGCGACGGAGTGAATGAAAAGGTGAGCTGTGTGTGACAGGCCAGTTGC
454  Q H S E D A S K D N S D G V N E K V S C V * 479

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Fig. 2. The nucleotide sequence and deduced amino acid sequence encoding the rhesus monkey 5-HT_{2A} receptor. Sequences were isolated from a cDNA λ library using a radiolabelled rat 5-HT_{2A} probe and low stringency hybridization.

affinity to the human 5-HT_{2A} receptor. Admittedly, with most 5-HT_{2A} ligands there is no difference in affinity for the rat and human receptors and to date the only 5-HT_{2A} ligands that have shown species differences are of a specific structural type (i.e., indole containing compounds). However, the fact that some compounds do show species differences between the rat and the pig, squirrel monkey, rhesus monkey and human, and that the structure–activity relationship actually reverses between these species [12,13] means that the results from rat cannot be used with a high degree of confidence to predict activity in humans. Furthermore, if the affinity of a compound for one receptor (for example the 5-HT_{2A} receptor) changes between species it does not necessarily follow that the affinity of the compound will change for any other receptor (for example the 5-HT_{2C} receptor). Therefore, one may have a decreased or even a loss of selectivity for a target resulting in an increase chance for side effects in man.

With the 5-HT_{2A} receptor it is now clear that the species differences observed in affinities for ergolines and tryptamines is due to a single amino acid substitution,

Ala/Ser-242 [16,17]. However, there are two other amino acid substitutions within the TM regions of the rat and the pig, rhesus monkey and human 5-HT_{2A} receptors. The Ile-150 in the rat to Val-150 in the higher species is less likely to result in significant changes in the ability of compounds to bind to these receptors. However, the rat 5-HT_{2A} receptor contains a Thr-82 while the pig, rhesus monkey and human 5-HT_{2A} receptors have an Ala in this position. It is easy to envision an H-bonding interaction occurring in the rat if this Thr were positioned in the appropriate site within the binding pocket. Naturally this H-bonding interaction would be lost in the pig, rhesus monkey and human 5-HT_{2A} receptors. Although no such compounds have been identified, it is possible that compounds other than the ergolines and tryptamines could potentially show species differences in their affinity for the 5-HT_{2A} receptor.

One obvious solution to this potential problem of species specific affinities is to utilize rhesus monkey frontal cortex or the pig frontal cortex, or the pig, rhesus monkey or human cloned receptor, when determining affinity for the

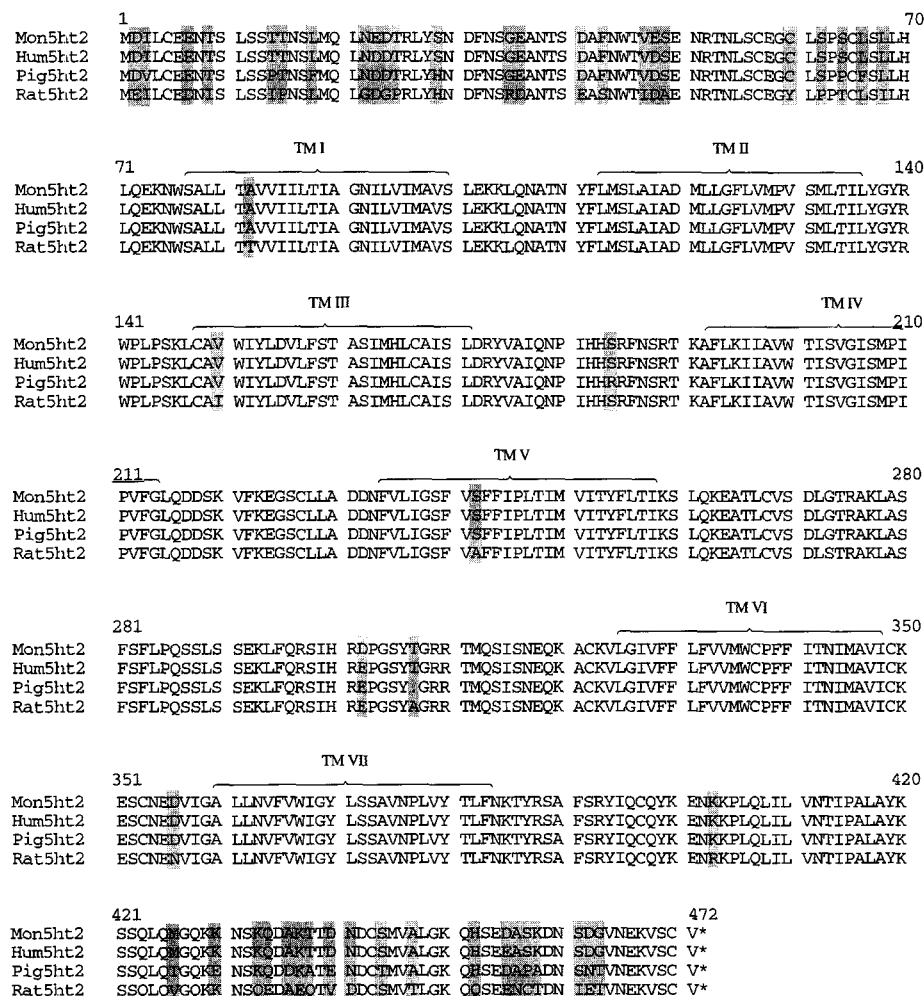


Fig. 3. Multiple alignment comparison of deduced amino acid sequences from the human, pig, rhesus monkey and rat 5-HT_{2A} receptors. Shaded areas indicate species differences in the amino acid sequence.

5-HT_{2A} receptor. Previous work [12,13,17] has clearly shown that those compounds that do show species differences in affinity have a similar affinity for the pig, rhesus monkey and human 5-HT_{2A} receptors suggesting that the rhesus monkey and pig serve as appropriate models for affinity to the human 5-HT_{2A} receptor. The present report greatly strengthens this argument by deducing the amino acid sequence of the pig and rhesus monkey 5-HT_{2A} receptors from cDNA clones. The fact that the pig, rhesus monkey and human 5-HT_{2A} receptors have identical amino acid sequences within the TM regions and that all the modeling and mutagenesis work to date indicate that 5-HT_{2A} receptor ligands bind within a pocket formed by the TM regions strongly suggests no compounds will be found that bind with a different affinity to the pig, rhesus monkey and human 5-HT_{2A} receptors. Therefore, either the rhesus monkey or the pig 5-HT_{2A} receptors could serve as more reasonable models to predict affinity to the human 5-HT_{2A} receptor.

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