Molecular determinants of ligand binding to H_4R species variants

Herman D. Lim, Chris de Graaf, Wen Jiang, Payman Sadek, Patricia M. McGovern, Enade P. Istyastono, Remko A. Bakker, Iwan J.P. de Esch, Robin L. Thurmond, and Rob Leurs

Supplementary information

Contents:

Supplementary Table I. *Affinity (pK_i) of H_4R ligands at the human, pig, and dog H_4Rs and selected human H_4R mutants. Equilibrium dissociation constants (K_D) and B_{max} values for [^{3}H]histamine (pmol/mg protein) and pK_i of H_4R ligands are presented as average ± standard error of mean (SEM) of results of at least three independent experiments.*

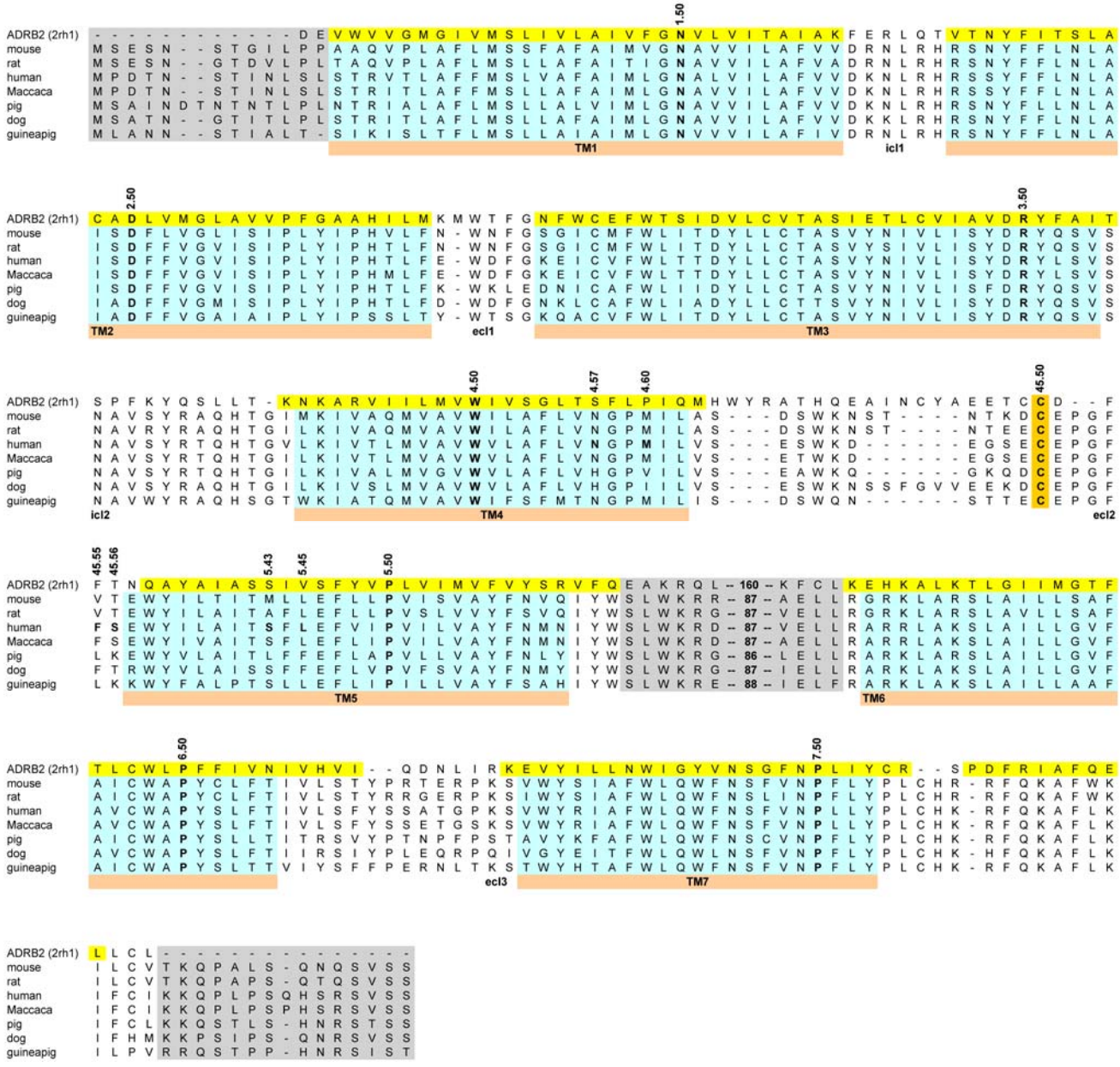
Receptor	[^{3}H]histamine		pK_i			
	K_D (nM)	B_{max}	Histamine	Clozapine	JNJ 7777120	VUF 8430
Human H_4R	9 ± 1	3.1 ± 1.1	7.9 ± 0.1	6.4 ± 0.1	8.3 ± 0.1	7.5 ± 0.1
Pig H_4R	11 ± 3	1.0 ± 0.3	7.8 ± 0.1	5.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1
Dog H_4R	75 ± 14	3.7± 0.8	6.9 ± 0.1	4.5 ± 0.1	7.1 ± 0.1	5.9 ± 0.1
mkH4R	15 ± 2	6.9 ± 0.7	7.8 ± 0.1	7.2 ± 0.1	7.4 ± 0.1	7.3±0.1
PHP chimera	4 ± 1	0.3 ± 0.1	8.3 ± 0.1	6.8 ± 0.1	7.8 ± 0.1	7.4 ± 0.1
HPH chimera	18 ± 2	2.9 ± 0.2	7.8 ± 0.1	4.7 ± 0.1	6.1 ± 0.1	6.3 ± 0.2
N4.57H	51 ± 5	2.2 ± 0.5	7.4 ± 0.1	4.7 ± 0.1	7.7 ± 0.1	6.8 ± 0.1
M4.60V	14 ± 3	2.4 ± 0.2	7.8 ± 0.1	6.2 ± 0.1	8.2 ± 0.1	7.2 ± 0.1
$S45.42^{156A}$	6 ± 1	0.2 ± 0.1	7.8 ± 0.1	6.5 ± 0.1	8.2 ± 0.1	7.5 ± 0.1
$F45.55^{169L}$	18 ± 2	2.9 ± 0.3	7.8 ± 0.1	5.7 ± 0.1	8.4 ± 0.1	7.3 ± 0.1
$F45.55^{169}L/S45.56^{170}K$	8 ± 1	3.5 ± 1.4	7.8 ± 0.1	5.6 ± 0.1	8.3 ± 0.1	7.6 ± 0.1
I5.38V	10 ± 2	3.5 ± 0.6	7.9 ± 0.1	6.2 ± 0.1	8.3 ± 0.1	7.4 ± 0.1
L5.39V	10 ± 3	3.5 ± 0.6	8.0 ± 0.1	7.0 ± 0.1	7.1 ± 0.1	n.t.[a]
S5.43L	12 ± 2	3.3 ± 0.4	7.7 ± 0.1	6.6 ± 0.1	7.8 ± 0.1	7.3 ± 0.1
L5.45F	17 ± 2	3.0 ± 0.3	7.8 ± 0.1	6.1 ± 0.1	8.3 ± 0.1	7.0 ± 0.1
N4.57H/S5.43L	15 ± 3	1.9 ± 0.3	7.8 ± 0.1	4.9 ± 0.1	7.5 ± 0.1	6.5 ± 0.1

[a] Not tested

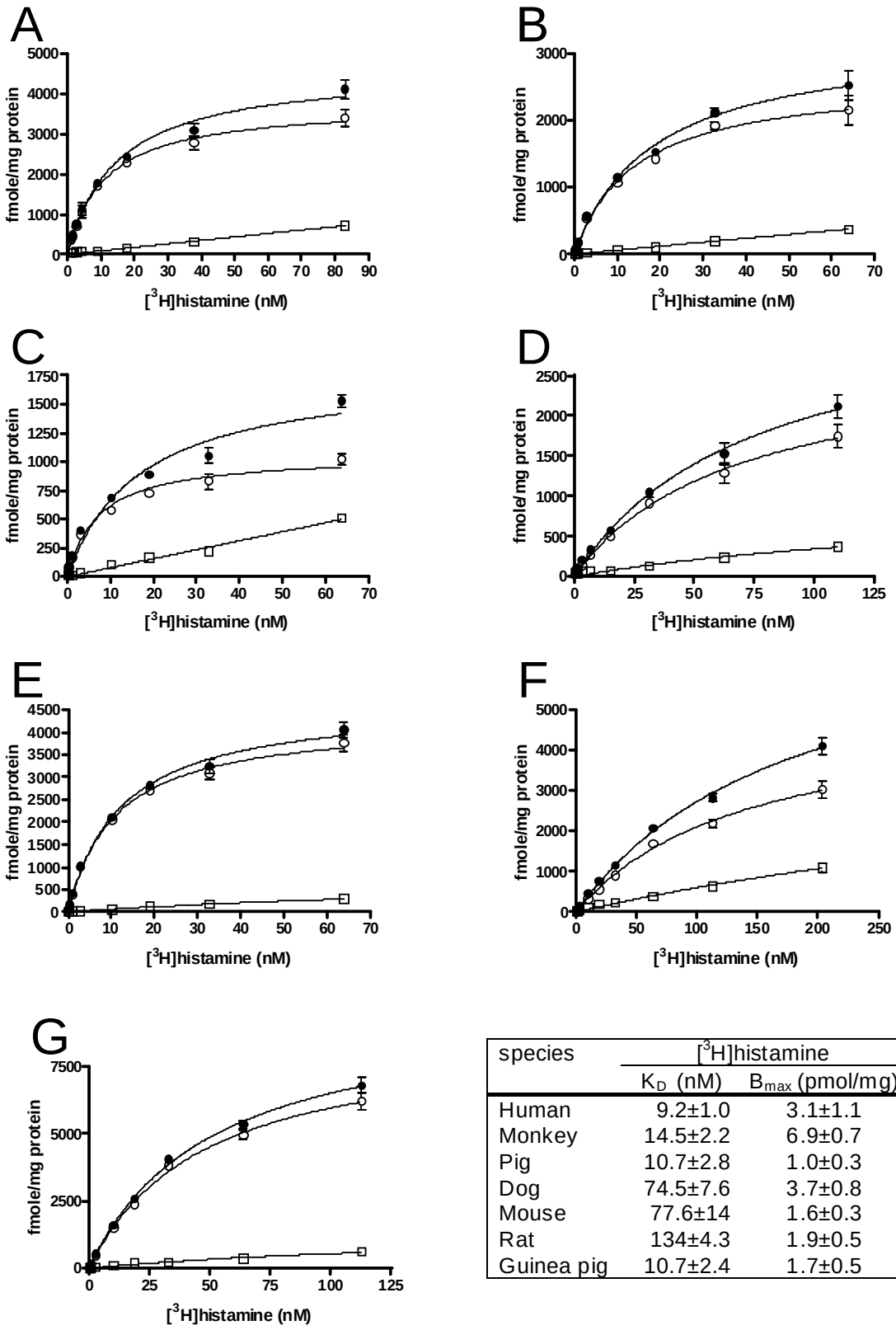
Supplementary Table II. *Potencies (pEC_{50}) of histamine at the H_4 receptor variants are presented as average ± standard error of mean (SEM) of results of at least three independent experiments.*

	histamine pEC_{50}
human	7.00±0.08
monkey	6.97±0.12
pig	6.59±0.09
dog	6.87±0.04
mouse	6.23±0.09
rat	6.04±0.16
guinea pig	6.45±0.09

Supplementary Figure I. *Sequence alignment of the human adrenergic beta2 receptor (ADRB2) crystal structure template (boxed in yellow) to human, mouse, rat, maccaca, pig, dog, guineapig histamine H4 receptor. Transmembrane domains (TM1 to TM7) as well as helix 8 (H8) are boxed in cyan. Ecl1-3 and icl1-3 indicate the positions of extracellular and intracellular loops. Residues in bold are conserved Ballesteros-Weinstein reference residues. The residues referred to in the text of the current paper are indicated by their number according to Ballesteros-Weinstein and residue numbers in ecl2 as defined in the Methods section of the current paper. Numbers inserted in the icl3 loop describe the number of residues omitted in the current study (boxed in grey).*

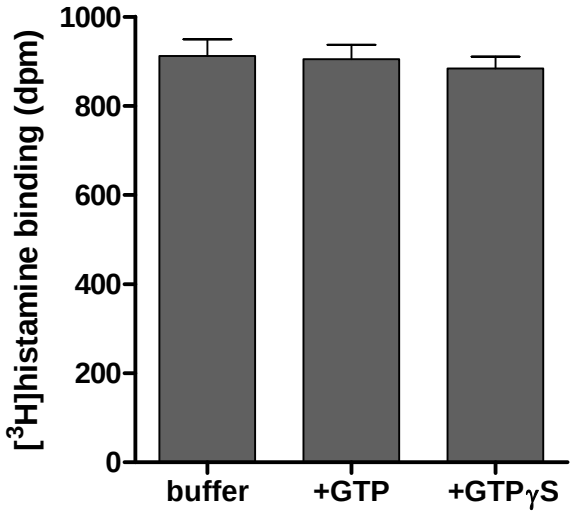


***Supplementary Figure II.**: Saturation binding of [^{3}H]histamine at the H_4R orthologs of human (**A**), monkey (**B**), pig (**C**), dog (**D**), rat (**E**), guinea pig (**F**), and mouse (**G**). Non-specific binding was determined with 1-30 μM JNJ 7777120. Total binding is depicted in black circles, specific binding in open circles, and non-specific binding in open square.*



species	[^{3}H]histamine	
	K_D (nM)	B_{max} (pmol/mg)
Human	9.2±1.0	3.1±1.1
Monkey	14.5±2.2	6.9±0.7
Pig	10.7±2.8	1.0±0.3
Dog	74.5±7.6	3.7±0.8
Mouse	77.6±14	1.6±0.3
Rat	134±4.3	1.9±0.5
Guinea pig	10.7±2.4	1.7±0.5

Supplementary Figure III. *Binding of [^{3}H]histamine to the human H_4R expressed in HEK 293T cells in the absence and presence of 10 μM GTP or GTPγS. The error bars indicate the standard error of mean (SEM) of results of at least three independent experiments.*



Supplementary Figure IV. *Functional responses of the H_4R orthologs to histamine in a $G\alpha_{qi5}$-directed NFAT-luciferase reporter gene assay according to metholodology described previously (Lim et al., 2008).*