ELSEVIER

European Journal of Pharmacology 369 (1999) 125–132

ejp

Identification of a domain affecting agonist potency of *meta*-chlorophenylbiguanide in 5-HT_3 receptors

Shinobu Mochizuki *, Akira Miyake, Kiyoshi Furuichi

Molecular Medicine Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical, 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan

Received 29 October 1998; revised 11 January 1999; accepted 26 January 1999

Abstract

The pharmacological properties of rat and human 5-HT_3 receptors expressed in *Xenopus* oocytes were assessed using a two-electrode voltage clamp technique. *Meta*-chlorophenylbiguanide (mCPBG), a 5-HT_3 receptor-selective agonist, elicited typical current responses in both rat and human 5-HT_3 receptor-expressing oocytes. However, the EC_{50} value for rat 5-HT_3 receptors was 13-fold lower than for human 5-HT_3 receptors. Using several chimeric human–rat 5-HT_3 receptors, we identified a potential domain responsible for this difference in mCPBG-response. The domain is in the N-terminal extracellular region adjacent to the first transmembrane domain of rat 5-HT_3 receptors and includes a rat-specific seven amino acid sequence (Phe^{197}, Thr^{198}, Lys^{199}, Gln^{201}, Ile^{205}, Thr^{207} and Ser^{210}). Replacement of corresponding amino acids in human 5-HT_3 receptors by rat receptor residues increased the potency of mCPBG on human receptors indicating these amino acids play an important role in the pharmacological response to mCPBG.

Keywords: 5-HT_3 receptor; Ion channel, ligand-gated; *Meta*-chlorophenylbiguanide; *Xenopus* oocyte

1. Introduction

5-HT_3 receptors are ligand-gated ion channels which cause fast, depolarizing responses in central and peripheral nervous system, and in neuronal cell lines (Yakel and Jackson, 1988; Derkach et al., 1989; Hoyer et al., 1994). cDNAs encoding 5-HT_3 receptors have been cloned from many mammalian species including mice, rats, guinea pigs, and humans (Maricq et al., 1991; Johnson and Heinemann, 1992; Isenberg et al., 1993; Belelli et al., 1995; Miyake et al., 1995; Lankiewicz et al., 1998). These cloned 5-HT_3 receptors form functional homo-oligomeric receptors when expressed in *Xenopus* oocytes or mammalian cells. The 5-HT_3 receptors have features of the ligand-gated ion channel family such as a large N-terminal extracellular domain and four hydrophobic transmembrane domains (M1–M4). They are most closely related to nicotinic acetylcholine receptors (Maricq et al., 1991). In addition, electron microscopic studies revealed that the quaternary structure of 5-HT_3 receptors is similar to that of nicotinic acetylcholine receptors (Unwin, 1993; Boess et al., 1995; Green et al., 1995).

The ligand binding domains of nicotinic acetylcholine receptors are studied using photoaffinity labeling experiments and mutagenesis of the receptors (Changeux et al., 1992). These studies found that the large extracellular N-terminal domain forms at least three loops (loops 1–3) that serve as the ligand binding domain. However, the ligand binding domains of 5-HT_3 receptors have not been examined directly because of the lack of effective ligands. Interestingly, chimeric receptors formed from 5-HT_3 receptors and α7 neuronal nicotinic acetylcholine receptors indicate that agonist specificity for 5-HT_3 and nicotinic acetylcholine receptors depend on their N-terminal extracellular domain (Eiselé et al., 1993). However, these experiments did not sufficiently examine the details of ligand-receptor interaction in 5-HT_3 receptors.

Previously, we found that the binding affinity of the 5-HT_3 receptor selective agonist mCPBG for rat 5-HT_3 receptors was about 100 times higher than for human 5-HT_3 receptors, whereas the affinities of other ligands are almost parallel (Miyake et al., 1995). Therefore, this study probed the N-terminal portions of rat and human 5-HT_3

* Corresponding author. Tel.: +81-298-52-5111; Fax: +81-298-52-5444; E-mail: mochizuk@yamanouchi.co.jp

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00058-8

receptors using pharmacological and molecular biological techniques to determine which domains affect mCPBG affinity. We constructed chimeric human–rat 5-HT_3 receptors and mutant human 5-HT_3 receptors, expressed the receptors in *Xenopus* oocytes, and determined a domain which contributes to the potency of mCPBG.

2. Materials and methods

2.1. Mutagenesis

Chimeric and mutant 5-HT_3 receptor used in this study are illustrated in Figs. 1 and 2.

The HR221, HR135 and HR196 chimeric 5-HT_3 receptors had the human N-terminal extracellular domain followed by the rat 5-HT_3 receptor. The HR221 chimeric receptor cDNA was generated using the naturally occurring *Nae*I site at the same relative position in human and rat receptor cDNAs (human: nucleotides 935–940 in the GenBank D49394; rat: nucleotides 731–736 in the GenBank D49395). The HR135 chimera was constructed using the *Bst*EII site and the HR196 chimera was constructed using the *Apo*I site, both of which exist only in rat receptor cDNA (nucleotides 474–480 and 664–669, respectively). The *Bst*EII site or *Apo*I site in human receptor was introduced into the equivalent position by polymerase chain reaction using a primer containing the restriction site. To construct chimera HR135, the primer was designed to introduce a mutation (nucleotide T684C in the human receptor) which did not change the amino acid. To construct chimera HR196, the primer was designed not only to introduce *Apo*I site but also to convert the LPY (human: amino acid positions 192–194) to FTK (rat: amino acid positions 197–199).

Amino acid changes in human 5-HT_3 receptor cDNAs were introduced using the polymerase chanin reaction-based mutagenesis technique (Tomic et al., 1990). Mutations introduced in human 5-HT_3 receptor were as follows (numbers refer to amino acid positions in the human 5-HT_3 receptor amino acid sequence): HR7 (L192F, P193T, Y194K, R196Q, M200I, S202T and Y205S), HR4 (L192F, P193T, Y194K and R196Q), HR3 (M200I, S202T and Y205S), HR2 (Y194K and R196Q).

All mutations were verified by DNA sequencing.

2.2. Functional expression in Xenopus oocytes

Capped cRNAs for 5-HT_3 receptors were transcribed in vitro from linearized plasmids containing the appropriate cDNA inserts as described previously (Miyake et al., 1995). *Xenopus laevis* oocytes were injected with cRNA. Following incubation for 1–3 days in modified Barth's medium (19°C), oocytes were treated with 2 mg/ml collagenase for 10–30 min to remove the follicular cell layers.

2.3. Electrophysiology and solutions

5-HT_3 receptor-mediated whole-cell currents were recorded from oocytes 1–14 days after collagenase treatment as described before (Miyake et al., 1995). Oocytes were voltage-clamped at a holding potential of −60 mV.

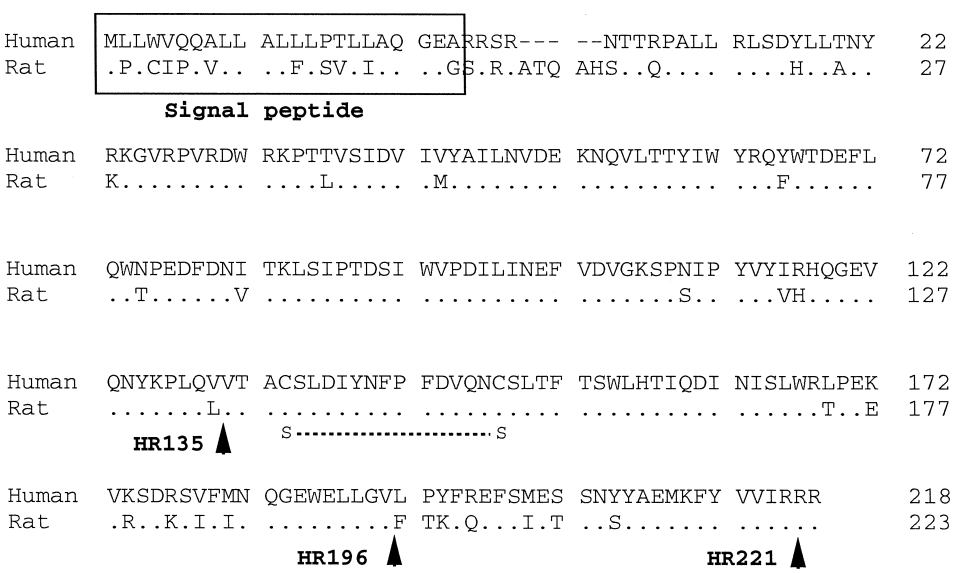

Fig. 1. Comparison of N-terminal extracellular domains of 5-HT_3 receptors. Amino acid sequences of human and rat 5-HT_3 receptors were aligned. Numbering of amino acid corresponds to the putative mature polypeptide. Arrowheads, switch point of HR221, HR135 and HR196 chimeric receptors; S-S, Cys–Cys loop. Open reading frames of human and rat 5-HT_3 receptor cDNA (human: GenBank D49394; rat: GenBak D49395) encoding a 478-amino acid and a 483-amino acid protein, respectively.

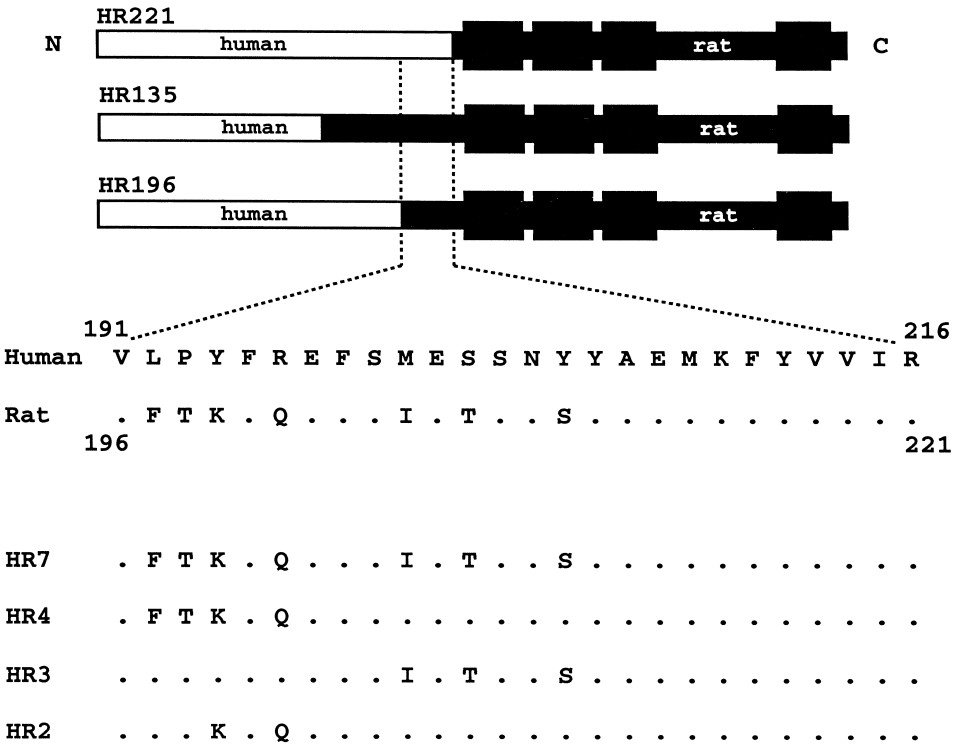

Fig. 2. Structure of chimeric and mutant 5-HT_3 receptors. Boxed areas represent putative transmembrane domains, N and C represent the amino- and carboxy-terminal domains. N-terminal amino acids (HR221: 1–221, HR135: 1–135, HR196: 1–196 amino acids) of rat 5-HT_3 receptors were replaced with corresponding regions of human 5-HT_3 receptors. HR7, HR4, HR3 and HR2 mutant receptors were human type 5-HT_3 receptors which contained mutations as follows (numbers refer to amino acid positions in the human 5-HT_3 receptor sequence): HR7 (L192F, P193T, Y194K, R196Q, M200I, S202T and Y205S), HR4 (L192F, P193T, Y194K and R196Q), HR3 (M200I, S202T and Y205S), HR2 (Y194K and R196Q).

Microelectrodes were filled with 3 M KCl and had resistances of 0.5–4 MΩ. Defolliculated oocytes were continuously perfused with bathing solution (112 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, and 10 mM HEPES, pH 7.2) at a rate of approximately 5 ml/min. Ion currents were elicited by switching to agonist-containing bathing solutions. Oocytes were repetitively exposed to agonists at intervals of at least 8 min in order to allow complete recovery from desensitization. mCPBG (Research Biochemicals) and 5-hydroxytryptamine creatinine sulphate (5-HT; Merck) solutions were freshly prepared in bathing solution. All experiments were performed at room temperature (25°C).

2.4. Data analysis

Concentration–response curves were fitted using the SAS program (SAS Institute, Japan) to the Hill equation:

$$\frac{i}{i_{max}} = \frac{[A]^{n_H}}{[A]^{n_H} + [EC_{50}]^{n_H}}$$

where A is agonist concentration, i_{max} is the maximum inward current evoked by a saturating concentration of agonist, i is inward current, EC_{50} is the concentration of agonist evoking a half-maximal response, and n_H is the Hill coefficient.

3. Results

mCPBG elicited 5-HT_3 receptor-mediated typical current responses in human and rat 5-HT_3 homo-oligomeric receptors expressed in *Xenopus* oocytes (Fig. 3A). The concentration–response curves for mCPBG indicated that the EC_{50} value for rat 5-HT_3 receptors (mean = 0.20 μM, 95% confidence interval = 0.15–0.26 μM; $n = 3$) was 13-fold lower than that for human 5-HT_3 receptors (2.6 μM, 2.4–2.8 μM; $n = 5$). Similarly, the n_H value for rat 5-HT_3 receptors (mean = 1.1, 95% confidence interval = 0.81–1.3; $n = 3$) was lower than that for human 5-HT_3 receptors (1.9, 1.7–2.1; $n = 5$; Fig. 3B, Table 1).

To identify regions of 5-HT_3 receptors responsible for mCPBG potency, we therefore systematically exchanged regions of the rat 5-HT_3 receptor with corresponding regions from the human 5-HT_3 receptor as shown in Figs. 1 and 2. First, we focused on the role of the N-terminal domain of the receptors. The N-terminal extracellular domain of rat 5-HT_3 receptors was substituted with the corresponding region from the human 5-HT_3 receptor (Figs. 1 and 2). The resulting chimeric receptor, HR221, responded to mCPBG in a concentration-dependent manner (Fig. 4A,B). The EC_{50} value for HR221 (mean = 2.9 μM, 95% confidence interval = 2.5–3.3 μM; $n = 3$–5) was very close to that for human 5-HT_3 receptors (Fig. 4B,

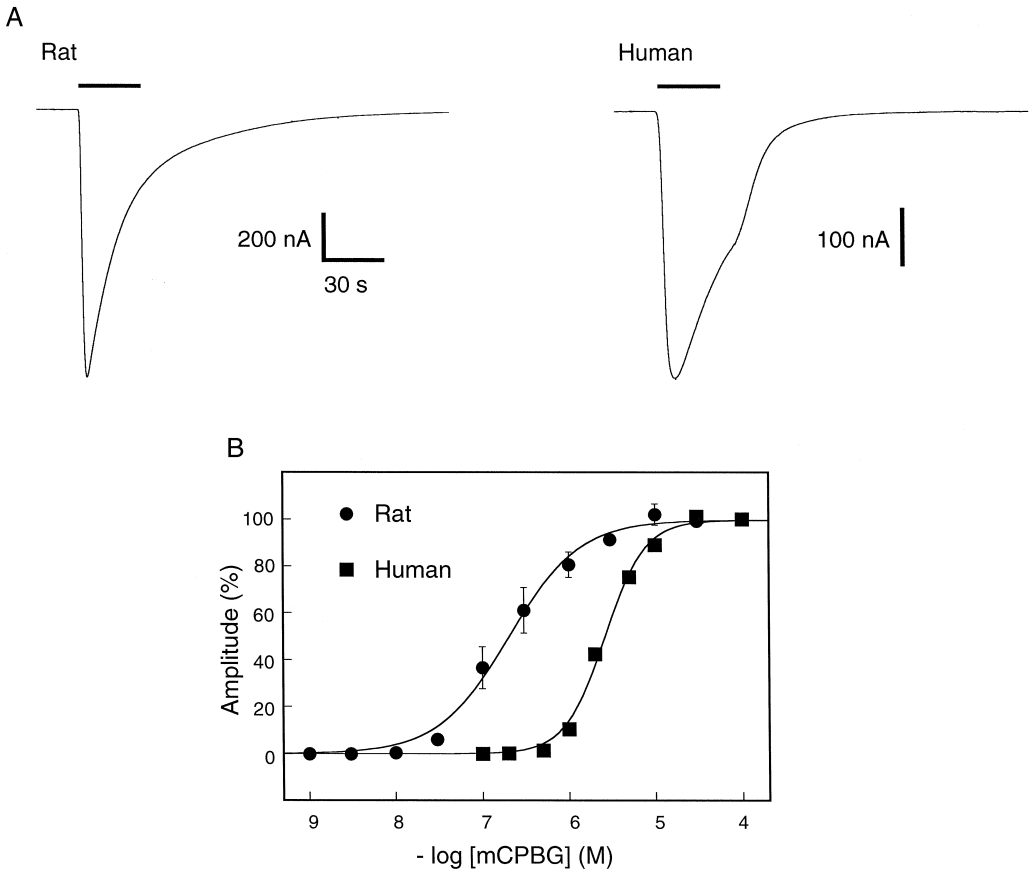

Fig. 3. Functional expression of rat and human 5-HT_3 receptors. (A) Typical responses elicited by bath applied 10 μM mCPBG (indicated by the horizontal bar) recorded at a holding potential of −60 mV. Rat and human 5-HT_3 receptors were expressed in *Xenopus* oocytes. (B) Concentration–response curves for mCPBG of rat and human 5-HT_3 receptors. Holding potential was −60 mV. Peak current amplitudes were normalized to 100 μM mCPBG-induced current. Each data point is the mean ± S.E.M. (n = 3–5). Absence of error bars indicates that the S.E.M. is smaller than the symbol size. Curves were fitted to the points as described in Section 2.

Table 1). This result suggests that the N-terminal extracellular domain of rat 5-HT_3 receptor is necessary for higher potency of mCPBG.

As shown in Fig. 1, there are many amino acids not conserved between rat and human 5-HT_3 receptors in the N-terminal extracellular domain. To narrow down candidates of amino acids responsible for mCPBG potency, amino acids 135 residues upstream of the Cys–Cys loop in rat 5-HT_3 receptor were swapped for corresponding human residues (Figs. 1 and 2) and mCPBG potency was analysed. mCPBG elicited current responses in this resulting chimeric receptor, HR135 (Fig. 4A). The concentration–response curve of HR135 (EC_{50}: mean = 0.58 μM, 95% confidence interval = 0.44–0.72 μM; n = 3) shifted leftward from that of human 5-HT_3 receptors (Fig. 4B, Table 1). Taken together with the finding about HR221, this result suggests that the region from the Cys–Cys loop to M1 affects mCPBG potency.

To define a boundary of possible amino acids responsible for mCPBG potency, another chimeric receptor HR196 was examined (Figs. 1 and 2). mCPBG elicited current responses in HR196 with an EC_{50} value (mean = 0.48 μM, 95% confidence interval = 0.37–0.58 μM; n = 5–6) lower than that for human 5-HT_3 receptors (Fig. 4A,B, Table 1). Thus, replacement of the N-terminal 196 amino acids in rat 5-HT_3 receptor with the corresponding human residues also increased potency of mCPBG. These results suggested that differences in the potency of mCPBG were

Table 1
Summary of mCPBG pharmacology for 5-HT_3 receptors expressed in *Xenopus* oocytes

Receptor	EC_{50} (μM)	n_H	Efficacy[a] (%)
Human	2.6 (2.4–2.8)	1.9 (1.7–2.1)	95 (88–102)
Rat	0.20 (0.15–0.26)	1.1 (0.81–1.3)	100 (92–108)
HR221	2.9 (2.5–3.3)	1.9 (1.5–2.3)	95 (93–97)
HR135	0.58 (0.44–0.72)	1.0 (0.81–1.2)	101 (94–109)
HR196	0.48 (0.37–0.58)	1.1 (0.89–1.3)	96 (87–105)
HR7	0.20 (0.17–0.23)	1.2 (1.0–1.4)	103 (92–114)
HR4	0.63 (0.54–0.72)	1.4 (1.2–1.7)	98 (92–103)
HR3	0.87 (0.69–1.0)	1.4 (1.1–1.7)	101 (95–107)
HR2	0.86 (0.75–0.97)	1.6 (1.3–1.9)	100 (97–102)

Values are means with 95% confidence intervals in parentheses obtained from three to six different oocytes.
[a]The peak amplitudes of the inward currents elicited by 100 μM mCPBG were expressed as a percentage of the currents elicited by 100 μM 5-HT.

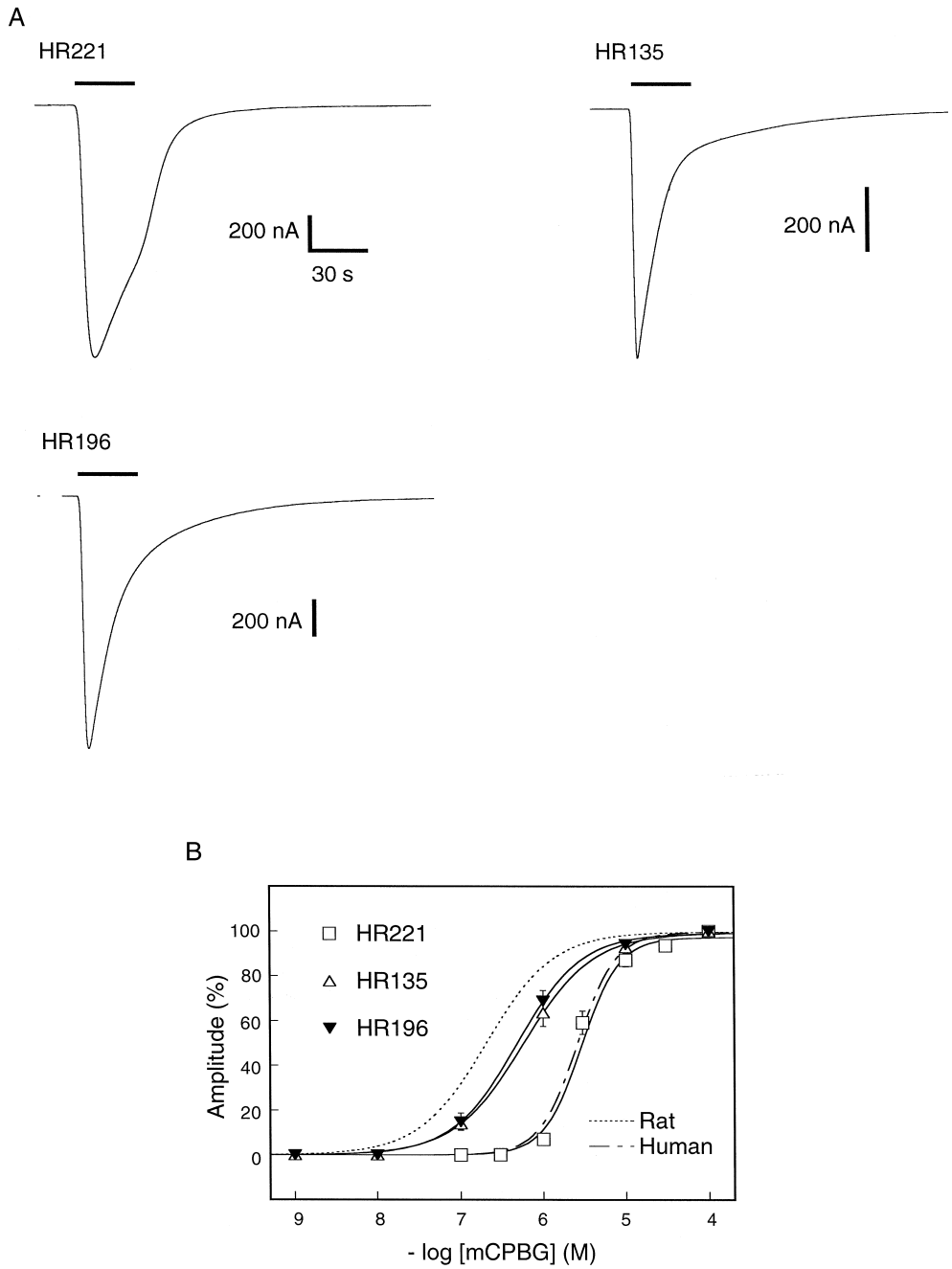

Fig. 4. Functional expression of HR221, HR135 and HR196 chimeric 5-HT_3 receptors. (A) Typical responses elicited by bath applied 10 μM mCPBG (indicated by the horizontal bar) recorded at a holding potential of −60 mV. Chimeric 5-HT_3 receptors were expressed in *Xenopus* oocytes. (B) Concentration–response curves for mCPBG of each chimeric 5-HT_3 receptor. Holding potential was −60 mV. Peak current amplitudes were normalized to 100 μM mCPBG-induced current. Each data point is the mean ± S.E.M. (n = 3–5). Absence of error bars indicates that the S.E.M. is smaller than the symbol size. Curves were fitted to the points as described in Section 2. Concentration–response curves of rat and human 5-HT_3 receptors from Fig. 3B are shown to facilitate the recognition of mCPBG potency on each receptor.

dependent on a 25-amino acid region located at the N-terminal domain adjacent to M1. The amino acid sequence in this region in rat 5-HT_3 receptors (F197–R221) was compared with the human 5-HT_3 receptor sequence. Only seven amino acids were unique to rat receptors: Phe^{197}, Thr^{198}, Lys^{199}, Gln^{201}, Ile^{205}, Thr^{207} and Ser^{210} (Figs. 1 and 2) and were therefore candidates as enhancers of mCPBG potency.

Next, we constructed a series of mutant human 5-HT_3 receptors (Fig. 2) to investigate which of the seven amino acids influence the potency of mCPBG. When all seven amino acids in human 5-HT_3 receptors were substituted with corresponding rat residues, the resulting mutant receptor HR7 responded to mCPBG and demonstrated high potency for mCPBG (EC_{50}: mean = 0.20 μM, 95% confidence interval = 0.17–0.23 μM; n = 3–4; Fig. 5A,B). This

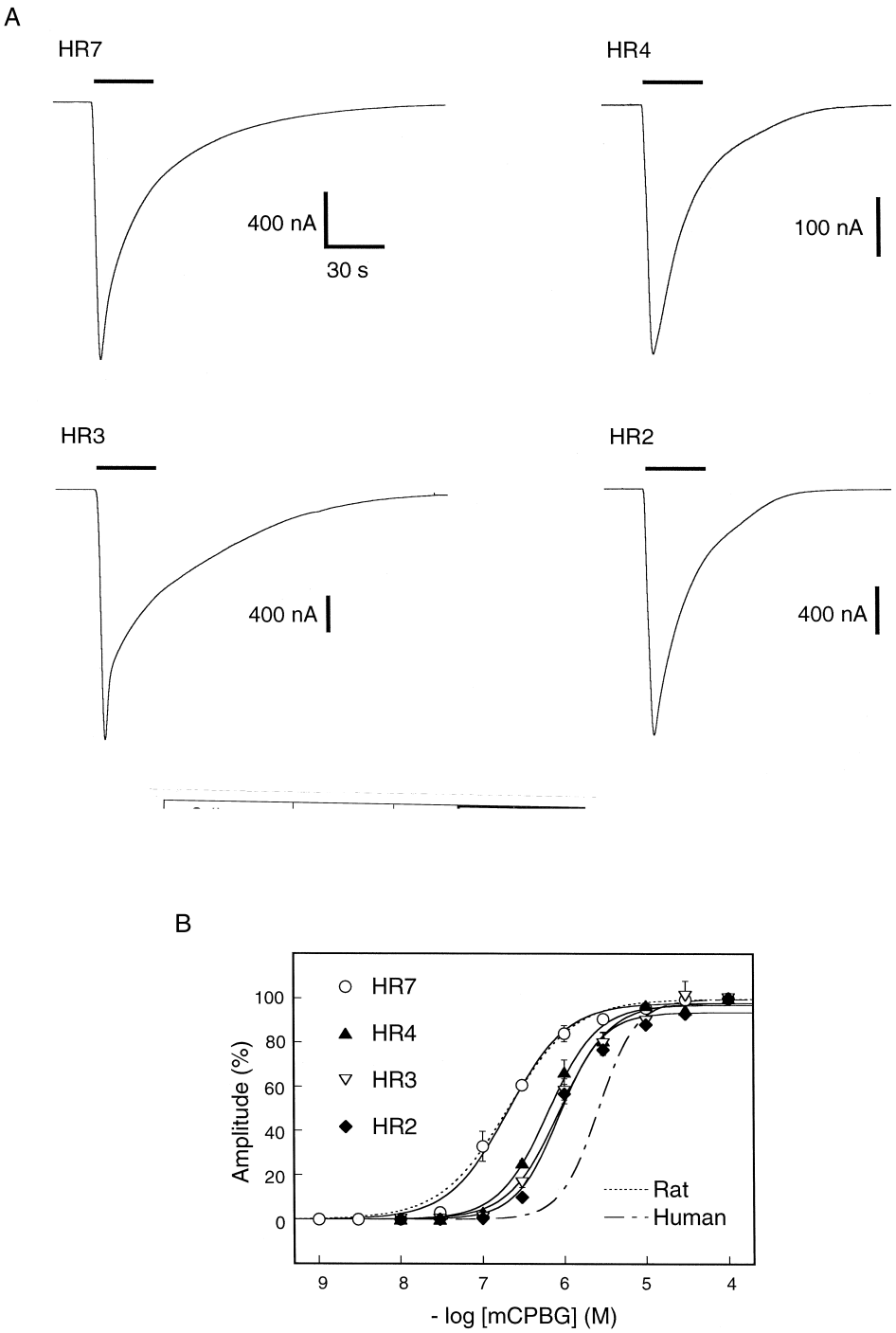

Fig. 5. Functional expression of HR7, HR3, HR4, and HR2 mutant 5-HT_3 receptors. (A) Typical responses elicited by bath applied 10 μM mCPBG (indicated by the horizontal bar) recorded at a holding potential of −60 mV. Mutant 5-HT_3 receptors were expressed in *Xenopus* oocytes. (B) Concentration–response curves for mCPBG of each mutant 5-HT_3 receptor. Holding potential was −60 mV. Peak current amplitudes were normalized to 100 μM mCPBG-induced current. Each data point is the mean ± S.E.M. ($n = 3–4$). Absence of error bars indicates that the S.E.M. is smaller than the symbol size. Curves were fitted to the points as described in Section 2. Concentration–response curves of rat and human 5-HT_3 receptors from Fig. 3B are shown to facilitate the recognition of mCPBG potency on each receptor.

potency is similar to that of rat 5-HT_3 receptors (Table 1), indicating that all seven amino acids from the rat 5-HT_3 receptor increase mCPBG potency, at least in the region bounded by F197–R221 (rat). When only four (HR4) or two (HR2) rat amino acids in N-terminal half, or three (HR3) rat amino acids in C-terminal half, of those seven amino acids were substituted in the human 5-HT_3 receptor, all concentration–response curves for mCPBG of these mutant receptors shifted leftward from that of human 5-HT_3 receptors (Fig. 5B). However, the mCPBG potency

for the HR2, HR3 and HR4 mutant receptors was not nearly as high as HR7 (Fig. 5B, Table 1). These results suggest that if a portion of these seven amino acids are substituted, mCPBG potency is partially enhanced.

Furthermore, the n_H values of HR135, HR196 and HR7 receptors were more similar to the rat 5-HT_3 receptor, while the value of the HR221 receptor was more similar to the human 5-HT_3 receptor. The other mutant receptors demonstrated n_H values between rat and human 5-HT_3 receptors. These results suggest that the region which affects the EC_{50} values for mCPBG also affects the n_H values.

The peak amplitudes of the inward currents elicited by a saturating concentration of mCPBG (100 μM) as compared to the amplitudes of the currents elicited by a saturating concentration of 5-HT (100 μM, data not shown) were similar for all 5-HT_3 receptors tested (Table 1). mCPBG was a full agonist on those receptors (Table 1).

4. Discussion

The EC_{50} value of mCPBG for rat 5-HT_3 receptors was 13-fold lower than that for human 5-HT_3 receptors when expressed in *Xenopus* oocytes. The value for rat 5-HT_3 receptors was comparable to a recently reported value by Mair et al. (1998). Furthermore, this result was qualitatively similar to our previously reported results which demonstrated that the binding affinity of mCPBG in rat 5-HT_3 receptors expressed in COS-1 cells was about 100 times higher than that of human 5-HT_3 receptors (Miyake et al., 1995). This quantitative discrepancy might be due to methodological reasons, because binding parameters were obtained from equilibrium reactions in prolonged exposure of ligands (Sepúlveda et al., 1991; Boess et al., 1997).

Analysis of mCBPBG potency using chimeric rat–human 5-HT_3 receptors suggests that seven amino acids in the N-terminal extracellular domain of 5-HT_3 receptors play an important role in determining mCPBG potency. Indeed, mutant human 5-HT_3 receptors, in which all seven amino acids were replaced with corresponding rat residues, had EC_{50} values for mCPBG quite similar to rat 5-HT_3 receptors.

The ligand binding domain of nicotinic acetylcholine receptors, which exhibits sequence similarity to 5-HT_3 receptors, are composed of at least three different domains, termed loops 1–3, in the N-terminal extracellular domain (Changeux et al., 1992). The 5-HT_3 receptor region containing the seven amino acids roughly corresponds to the loop 3 domain of nicotinic acetylcholine receptors. Consequently, it is strongly suggested that those seven amino acids play an important role in mCPBG binding. However, it is still unclear whether these amino acids are involved in direct interaction with mCPBG or influence the binding affinities of mCPBG through conformational changes of 5-HT_3 receptors. In addition, lower values of n_H of rat, HR135, HR196 and HR7 receptors than those of human and HR221 receptors suggest that this putative loop 3 domain of 5-HT_3 receptors may participate in an inter-subunit cooperativity (Boess et al., 1997).

Recently, Lankiewicz et al. (1998) demonstrated the importance of the putative loop 3 domain in 5-HT_3 receptors. Using chimeric human-guinea pig 5-HT_3 receptors, they reported that the 28 amino acids adjacent to M1 were essential to agonist (1-phenylbiguanide) selectivity. In this 28 amino acid region, there are several amino acid variations between human, guinea pig, mouse and rat 5-HT_3 receptors (Lankiewicz et al., 1998). Additionally, differences in the antagonist potency of (+)- tubocurarine between human and mouse 5-HT_3 receptors were partly influenced by the putative loop 3 domains (Hope et al., 1996, 1997). These reports also suggest that structural variations in putative loop 3 domains participate in pharmacological differences of 5-HT_3 receptors.

Amino acids sequences of T88–P114 and S154–R173 in rat 5-HT_3 receptors, which possibly include putative loops 1 and 2 domain respectively, are the same as those of corresponding human residues (Miyake et al., 1995). Therefore, this study could not exclude the possibility of mCPBG binding to these putative binding domains as well (Boess et al., 1997).

Our data show that chimeric receptors are important tools in elucidating differences in pharmacological responses between species. Further study of these differences, especially between human and other species, will help in developing 5-HT_3 receptor-selective drugs.

Acknowledgements

We would like to thank Drs. Gensei Kon and Toshiyuki Takemoto for support of this research. We also thank Mr. Steven E. Johnson for editing the manuscript.

References

Belelli, D., Balcarek, J.M., Hope, A.G., Peters, J.A., Lambert, J.J., Blackburn, T.P., 1995. Cloning and functional expression of a human 5-hydroxytryptamine type $3A_S$ receptor subunit. Mol. Pharmacol. 48, 1054–1062.

Boess, F.G., Beroukhim, R., Martin, I.L., 1995. Ultrastructure of the 5-HT_3 receptor. J. Neurochem. 64, 1401–1405.

Boess, F.G., Steward, L.J., Steele, J.A., Liu, D., Reid, J., Glencorse, T.A., Martin, I.L., 1997. Analysis of the ligand binding site of the 5-HT_3 receptor using site directed mutagenesis: importance of glutamate 106. Neuropharmacology 36, 637–647.

Changeux, J.-P., Devillers-Thiéry, A., Galzi, J.-L., Bertrand, D., 1992. New mutants to explore nicotinic receptor functions. Trends Pharmacol. Sci. 13, 299–301.

Derkach, V., Surprenant, A., North, R.A., 1989. 5-HT_3 receptors are membrane ion channels. Nature 339, 706–709.

Eiselé, J.L., Bertrand, S., Galzi, J.L., Devillers-Thiéry, A., Changeux, J.P., Bertrand, D., 1993. Chimaeric nicotinic-serotonergic receptor

combines distinct ligand binding and channel specificities. Nature 366, 479–483.

Green, T., Stauffer, K.A., Lummis, S.C.R., 1995. Expression of recombinant homo-oligomeric 5-hydroxytryptamine$_3$ receptors provides new insights into their maturation and structure. J. Biol. Chem. 270, 6056–6061.

Hope, A.G., Belelli, D., Lambert, J.J., Peters, J.A., 1996. Structural determinants of the antagonist potency of (+)-tubocurarine at recombinant 5-HT$_3$ receptor subunits. Br. J. Pharmacol. 119, 292P.

Hope, A.G., Belelli, D., Mair, I.D., Muntoni, A.L., Peters, J.A., Lambert, J.J., 1997. The structural determinants of the antagonist potency of (+)-tubocurarine at recombinant 5-HT$_3$ receptor subunits. Soc. Neurosci. Abst. 23, 150.6.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Isenberg, K.E., Ukhum, I.A., Holstad, S.G., Jafri, S., Uchida, U., Zorumski, C.F., Yang, J., 1993. Partial cDNA cloning and NGF regulation of a rat 5-HT$_3$ receptor subunit. NeuroReport 5, 121–124.

Johnson, D.S., Heinemann, S.F., 1992. Cloning and expression of the rat 5-HT$_3$ receptor reveals species specific sensitivity to curare antagonism. Soc. Neurosci. Abst. 18, 249.

Lankiewicz, S., Lobitz, N., Wetzel, C.H.R., Rupprecht, R., Gisselmann, G., Hatt, H., 1998. Molecular cloning, functional expression, and pharmacological characterization of 5-hydroxytryptamine$_3$ receptor cDNA and its splice variants from guinea pig. Mol. Pharmacol. 53, 202–212.

Mair, I.D., Lambert, J.J., Yang, J., Dempster, J., Peters, J.A., 1998. Pharmacological characterization of a rat 5-hydroxytryptamine type$_3$ receptor subunit (r5-HT$_{3A(b)}$) expressed in *Xenopus laevis* oocytes. Br. J. Pharmacol. 124, 1667–1674.

Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M., Julius, D., 1991. Primary structure and functional expression of the 5HT$_3$ receptor, a serotonin-gated ion channel. Science 254, 432–437.

Miyake, A., Mochizuki, S., Takemoto, Y., Akuzawa, S., 1995. Molecular cloning of human 5-hydroxytryptamine$_3$ receptor: heterogeneity in distribution and function among species. Mol. Pharmacol. 48, 407–416.

Sepúlveda, M.-I., Lummis, S.C.R., Martin, I.L., 1991. The agonist properties of *m*-chlorophenylbiguanide and 2-methyl-5-hydroxytryptamine on 5-HT$_3$ receptors in N1E-115 neuroblastoma cells. Br. J. Pharmacol. 104, 536–540.

Tomic, M., Sunjevaric, I., Savtchenko, E.S., Blumenberg, M., 1990. A rapid and simple method for introducing specific mutations into any position of DNA leaving all other positions unaltered. Nucleic Acids Res. 18, 1656.

Unwin, N., 1993. Nicotinic acetylcholine receptor at 9 Å resolution. J. Mol. Biol. 229, 1101–1124.

Yakel, J.L., Jackson, M.B., 1988. 5-HT$_3$ receptors mediated rapid responses in cultured hippocampus and a clonal cell line. Neuron 1, 615–621.