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Cloning, expression and pharmacology of the mouse 5-HT_{4L} receptor

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Abstract Since most of our knowledge on pharmacological properties of brain 5-HT_4 receptors have been discussed for mouse colliculi neurons, we cloned the corresponding receptor using the RT-PCR approach. As expected, the homology with the already cloned rat 5-HT_{4L} receptor was high, revealing only 16 differences at the amino-acid level. One of the differences, proline[75] in mouse, alanine[75] in the already published rat sequences was not confirmed. Therefore this proline is part of the consensus sequence present in all 5-HT receptor transmembrane domain II (LVMP). Comparing the affinities of 11 agonists and five antagonists for the cloned mouse receptor (5-HT_{4L}) expressed in LLCPK1 and the corresponding receptor in mouse colliculi shows an excellent correlation. The transfected mouse 5-HT_{4L} receptor stimulated cAMP production. When expressed at high density, it exhibited intrinsic activity. In contrast to the previously described distribution, we found that mRNA encoding for both the short (5-HT_{4S}) and the long form (5-HT_{4L}) of 5-HT_4 receptors are expressed in all mouse and rat brain areas.

Key words: Mouse colliculi; 5-HT_4 receptor; Cloning; Distribution; Splice variant

1. Introduction

5-HT_4 receptors are expressed in a wide variety of tissues of vertebrates including rodent, porcine and human brain, guinea-pig ileum and colon, rat oesophagus, frog and human adreno-cortical cells, human urinary bladder, human and porcine heart [1–3]. In the alimentary tract, 5-HT_4 agonists such as metoclopramide and cisapride are therapeutically used as prokinetic drugs. In brain, a role for 5-HT_4 receptors in cognition is likely [1]. 5-HT_4 receptors were first described in mouse colliculi neurons, as a 5-HT receptor positively coupled to adenylyl cyclase (AC) and having a unique pharmacology, clearly different from that of 5-HT_1, 5-HT_2 and 5-HT_3 receptors [4]. Nowadays, 5-HT_4 pharmacology includes many highly specific antagonists such as GR 113808 and SB 204070 which are also excellent radioligands [5–8]. Most of the pharmacological and transductional studies on 5-HT_4 have been performed in mouse colliculi neurons, guinea-pig ileum, and rat oesophagus. Although the pharmacology of 5-HT_4 receptors in these preparations is very similar, some unexplained differences exist. For example, benzamides, are full agonists in colliculi neurons and partial agonists or antagonists in most other prepartions (guinea-pig ileum and distal colon, rat hippocampus) [2]. A difference in coupling efficiency is unlikely to explain these differences because in distal guinea pig colon, a preparation in which the potency for 5-HT is the highest, the benzamides are still partial agonists [9]. In addition, a recent report describes differences between pharmacology of the 5-HT_4 receptor in guinea pig colon and those present in rat oesophagus when novel 5-HT_4 compounds were used [10]. In order to understand these differences and in particular whether they correspond to species differences, to tissue differences or to coupling differences intrinsic to the receptor molecule, cloning of these receptors is necessary. So far, only 5-HT_4 receptors from rat brain have been cloned, a tissue from which we have very few functional studies. In this report we have cloned and studied the pharmacology of the 5-HT_{4L} receptor from mouse colliculi, a preparation in which most of our knowledge on 5-HT_4 receptor pharmacology and transduction processes has been established [3,11].

2. Materials and methods

2.1. Isolation and sequencing of the mouse 5-HT_4 cDNA by RT-PCR

Total RNA was isolated from colliculi neurons in primary culture prepared from 14–15-day-old Swiss mouse embryos [12]. Poly(A)$^+$ RNA was extracted with Dynabeads Oligo(dT)$_{25}$ (Dynal) and reverse transcribed using Superscript II Rnase H Reverse Transcriptase (Life Technologies) and random nonamers. RT-PCR were performed with oligonucleotides (ON), ON1–*Eco*RI (International Patent WO94/14957, Sequence 3, position 48–71) 5′-CGGAATTCTAATGGACAGACTTGATGCTAATG-3′, in the beginning of the 5′ coding sequence flanked with an *Eco*RI site, and ON2–*Xba*I (Patent, Sequence 3, position 1322–1340) 5′-GCTCTAGATTGCCTCATGCACTTGAA3′, in the 3′ non-coding sequence, flanked with an *Xba*I site. PCR was performed with the Vent DNA Polymerase (N.E. Biolabs): 94°C/3 min followed by 30 cycles of denaturation (94°C/30 s), annealing (54°C/30 s) and elongation (72°C/1 min 45 s). We used two successive PCR to have sufficient amplification. To confirm the sequence of the 5′ part of the receptor around the ATG codon, we performed a PCR reaction using the following primers: ON3 (Patent, Sequence 1, position 21–41) 5′-GGTTGGAAGGAGGAGGATGCT-3′, in the 5′ non-coding sequence, and ON4 (Patent, Sequence 1, position 648–670) 5′-GAGGAGAAACGGGATGTAGAAGG-3′, in the middle of the receptor sequence. The PCR conditions with Taq DNA Polymerase (Eurobio) were: 94°C/1 min followed by 35 cycles of denaturation (94°C/30 s), annealing (56°C/30 s) and elongation (72°C/1 min). Fragments (701 bp) were subcloned using the TA Cloning method (Invitrogen).

2.2. Tissue localisation studies

Brain areas were dissected from adult OFA rats, from new born (1 day) and adult Swiss mice. cDNA was obtained as described above from poly(A)$^+$ RNA. Primers designed to carry out PCR amplification were: ON1 for the two forms of the receptor and ON7 (Patent, Sequence 1, position 1227–1248) 5′- AGGGACTCTGGGTCATTGTGTA-3′ for the 5-HT_{4S} or ON2 for the 5-HT_{4L}, ON8 (J00691, position 2252–2271) 5′-GACTCCGGAGACGGGGTCAC-3′ and ON9 (J00691, position 2482–2501) 5′-CGCATCCTCTTCCTCCCTGG-3′ for β-actin. The PCR conditions were those described above for the Taq DNA Polymerase, with an annealing at 55°C. The expected sizes of the PCR fragments were 1150 bp for 5-HT_{4S}, 1293 for 5-HT_{4L} and 450 bp for β-actin. PCR products were run on 1% agarose/ethidium bromide gels and photographed. A positive control was performed with rat 5-HT_{4S} and L cDNA (a generous

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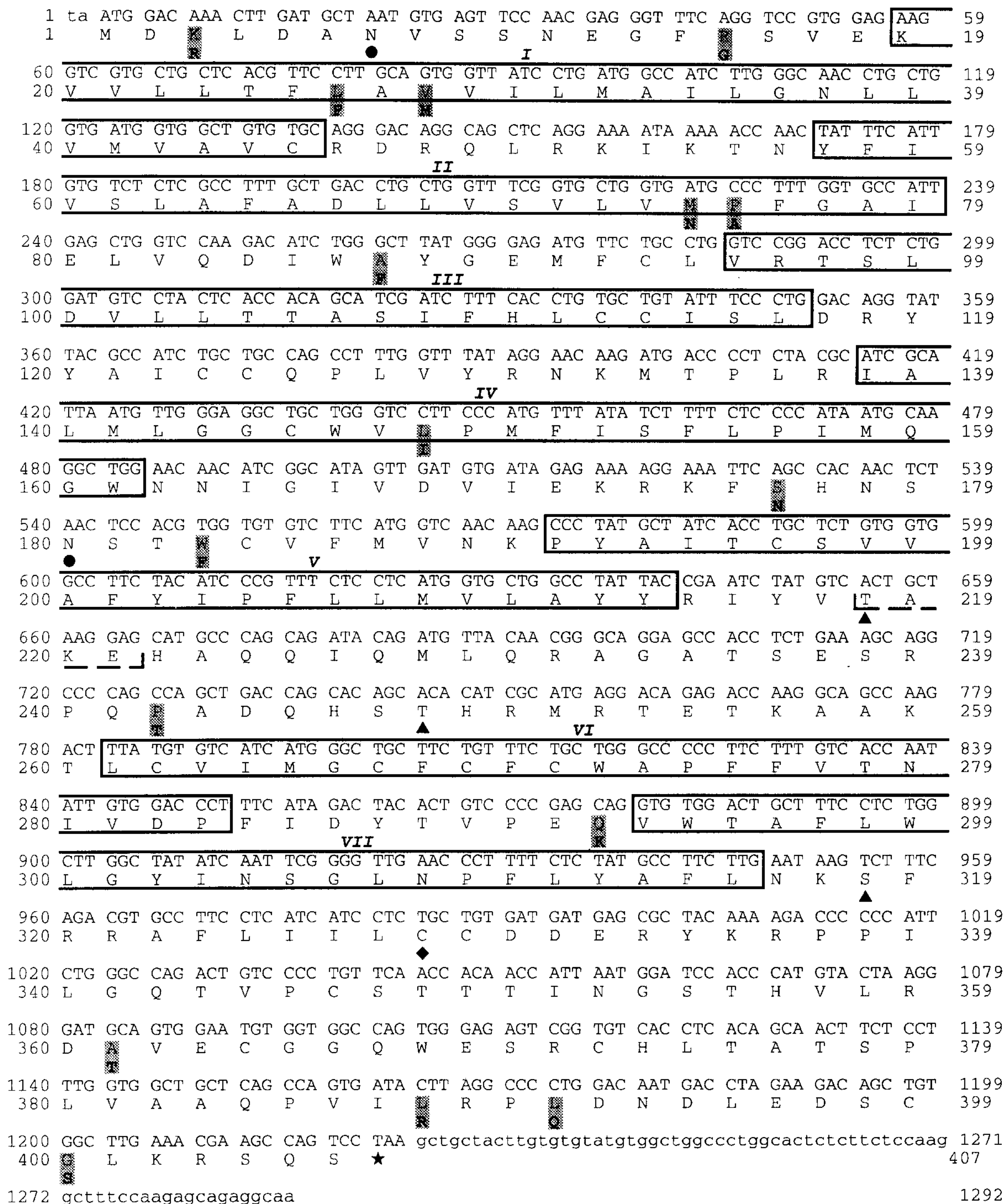

Fig. 1. Nucleotide sequence of the mouse 5-HT_{4L} cDNA. The receptor was sequenced on both strands from ON1 (position 1) to the end of ON2 (position 1292). The seven putative transmembrane domains are *boxed* and *numbered* (*I* to *VII*). Changes in mouse amino acids versus rat amino acids are *shaded* and rat amino acids are indicated under. ●, sites of potential N-linked glycosylation. ◆, site of potential palmitoylation. ▲, consensus sites for phosphorylation by protein kinase C. (— — —), consensus site of phosphorylation by calmoduline kinase 2. ★, terminal stop codon.

gift from Dr. Christophe Gerald). Southern blotting was done after transfer of the DNA to charged nylon membranes (Qiabond, Qiagen). The hybridization probe was an *Eco*RI–*Bam*HI fragment containing the 5′ non-coding region and the common region of the two 5-HT_4 forms to the *Bam*HI site (Patent, Sequence 1, position 1157) labeled using the Prime–ItII Random Primer Labeling Kit (Stratagene).

2.3. Transfection of LLCPK1 cells

5-HT_4 cDNAs were subcloned into pRK5, a CMV based expression vector [13] and introduced into LLCPK1 cells (a gift from Dr. Falk Fahrenholz, Germany) by electroporation. Briefly, cells were trypsinized, centrifuged, resuspended in EP1× buffer (50 mM K_2HPO_4/20 mM CH_3CO_2K/20 mM KOH/26.7 mM $MgSO_4$/pH 7.4) with 10–200 ng of receptor cDNA. After 15 min at room temperature, 300 μl of cell suspension (10^6 cells) were transferred to a 0.4 cm electroporation cuvette (Biorad) and pulsed using a Gene pulser apparatus (setting: 950 μF, 260 V). Cells were diluted in DMEM (10^6 cells/ml) containing 10% foetal bovine serum dialysed (FBSd) and plated on 10 cm Falcon petri dishes or into 12-well clusters.

2.4. Cyclic AMP assays

Six hours later, cells were incubated overnight with 2 μCi ^{3}H-la-

beled adenine to label the ATP pool and cAMP accumulation was measured as described [14].

2.5. Membrane preparations and radioligand binding assay

Membranes were prepared from transiently transfected LLCPK1 cells plated on 10 cm dishes and grown in DMEM with 10% FBSd for 6 h and 20 h in DMEM without FBSd. The cells were washed twice in PBS, scraped and harvested in the same buffer and centrifuged at 4°C, 900×g for 4 min. The cell pellet was resuspended in buffer containing 10 mM HEPES (pH 7.4), 5 mM EGTA, 1 mM EDTA and 0.32 M sucrose and homogenized with a glass-Teflon homogenizer at 4°C (10 shakes). The homogenate was centrifuged at 20,000×g for 20 min, the membrane pellet was resuspended in 50 mM HEPES (pH 7.4) (5 mg of protein in 1 ml of solution) and stored at −25°C until used for radioligand binding studies with ^{3}H-labeled GR 113808 (specific activity: 83 Ci/mmol) as previously described [8].

2.6. Data analysis

Competition and saturation experiments were analysed by non-linear regression using the LIGAND software [15].

3. Results

3.1. Cloning and primary amino acid sequence of the mouse 5-HT$_{4L}$ receptor

Recently, Gerald et al. [16] reported that the transcripts of the 5-HT$_{4L}$ form were expressed throughout the brain, whereas those of the short form (5-HT$_{4S}$) were limited to the mouse striatum. We performed RT-PCR experiments with poly(A)$^+$ RNA prepared from mouse embryo colliculi neurons using the primers (see Section 2) designed to clone the long form of the 5-HT$_4$ receptor (5-HT$_{4L}$). The resulting fragments (1293 bp) were double digested by *Eco*RI and *Xba*I and ligated into pRK5-neo. Double-stranded DNA was sequenced. The sequence was performed on three different clones from three independent PCR reactions. The sequence of the 5′ part of the receptor, around the ATG codon was controlled using another pCR fragment prepared with different oligonucleotides (see Section 2). Sequence analysis revealed one long open reading frame (406 amino acids) (Fig. 1). As expected, the homology with rat 5-HT$_4$ cDNA was high. However, 52 differences in the nucleotide sequence were present giving 16 differences in the amino-acid sequence. Compared to rat 5-HT$_{4L}$ receptor, note that the N-glycosylated site and most of consensus amino acids and sequences of biogenic amine receptors were conserved such as: the aspartates in transmembrane domains (TMD)-II (D66) and TMD-III (D100) engaged in the coupling to G protein and the ligand binding respectively, the cysteines of extracellular loops

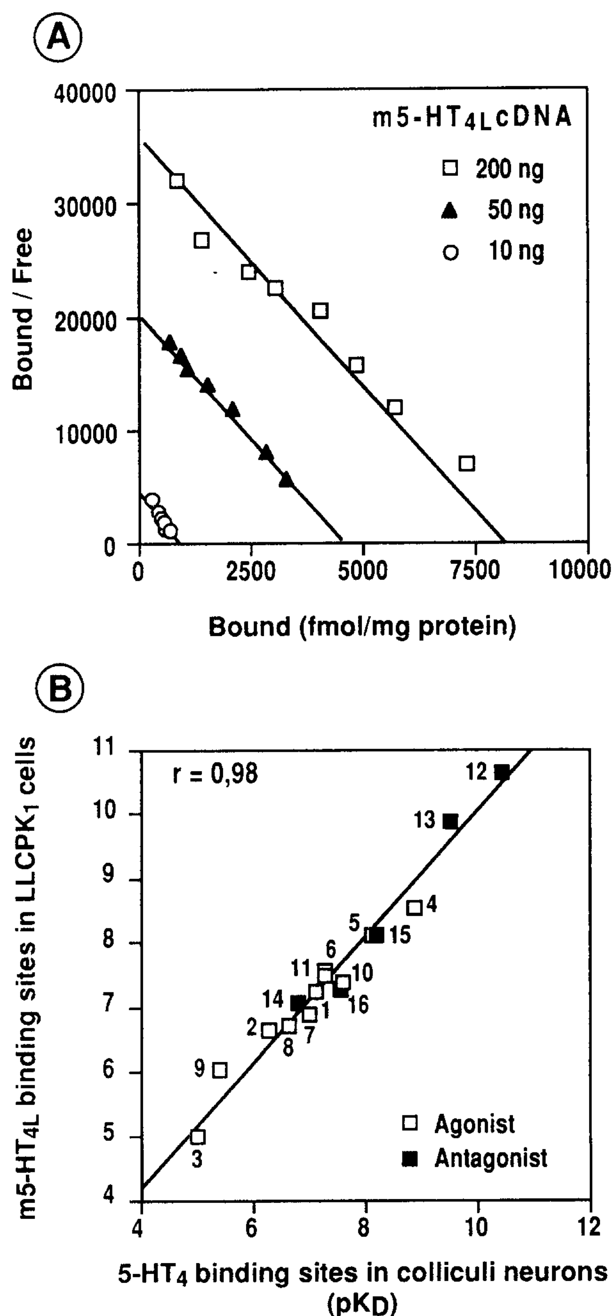

Fig. 2. A: Scatchard analysis of saturation experiments of ^{3}H-labeled GR 113808 binding to the mouse 5-HT$_{4L}$ receptors. Membranes (10–20 μg of protein/sample) harvested from transiently transfected LLCPK1 cells, electroporated with 10, 50, or 200 ng of mouse 5-HT$_{4L}$ receptor cDNA/10^6 cells, were incubated with 8 different concentrations of ^{3}H-labeled GR 113808 (0.01–1.8 nM) for 30 min at 20°C. B: Correlation between the affinities of 16 5-HT$_4$ drugs (11 agonists and 5 antagonists) for ^{3}H-labeled GR 113808 to the cloned mouse 5-HT$_{4L}$ receptors versus the affinities of the same drugs for ^{3}H-labeled GR 113808 binding sites in mouse colliculi neurons. Binding data corresponds to competition of ^{3}H-labeled GR 113808 binding to membranes of LLCPK1 cells transiently expressing mouse 5-HT$_{4L}$ receptors (750 fmol/mg protein). IC$_{50}$ values required to displace 50% of ^{3}H-labeled GR 113808 binding were determined experimentally and converted to K_d values according to the Cheng-Prussoff equation [29]: $K_d = IC_{50}/(1+S/K_{dS})$ where S is the ^{3}H-labeled GR 113808 concentration (0.2 nM) and K_{dS} the equilibrium constant of ^{3}H-labeled GR 113808. On the Y axis are reported the pKd values of 16 5-HT$_4$ drugs (see below) for cloned mouse 5-HT$_{4L}$ receptors and on the X axis, the pKd values of the same drugs for 5-HT$_4$ binding sites in colliculi neurons taken from [11]. For each drug the following values correspond to the pKd for cloned mouse 5-HT$_{4L}$ receptors and to the pKd for 5-HT$_4$ binding sites in colliculi neurons, respectively: (1) 5-HT: 7.25±0.12; 7.1±11; (2) 5-MeOT: 6.64±0.23; 6.28±0.17; (3) 5-CT: 5±0.12; 5.01±0.2; (4) RN 76186: 8.54±0.18; 8.86±0.05; (5) SC53116: 8.13±0.2; 8.1±0.08; (6) Cisapride: 7.49±0.09; 7.29±0.24; (7) Renzapride: 6.88±0.11; 7.01±0.12; (8) (S) Zacopride: 6.72±0.15; 6.65±0.15; (9) Metoclopramide: 6.05±0.13; 5.4±0.18; (10) BIMU 8: 7.39±0.23; 7.6±0.18; (11) BIMU 1: 7.54±0.16; 7.27±0.18; (12) GR 125487: 10.65±0.43; 10.42±0.10; (13) GR 113808: 9.69±0.32; 9.5±0.20; (14) ICS 205930: 7.06±0.35; 6.8±0.30; (15) SDZ 205557: 8.11±0.25; 8.2±0.22; (16) DAU 6285: 7.26±0.28; 7.57±0.15.

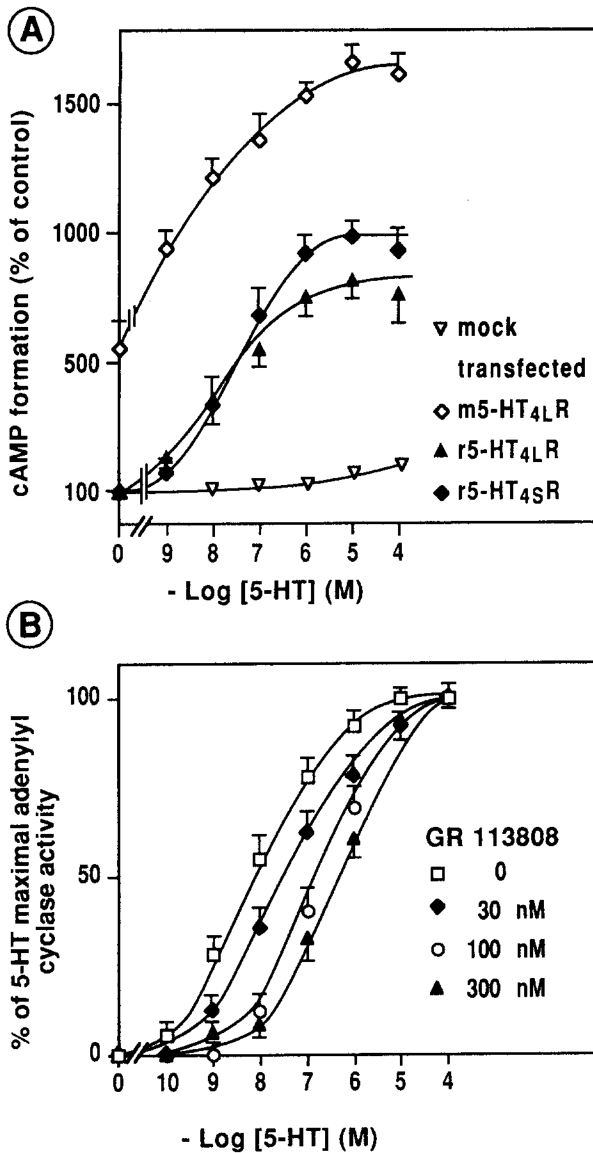

Fig. 3. A: Stimulation of cAMP production by increasing concentrations of 5-HT in LLCPK1 cells transiently expressing either the mouse 5-HT_{4L} or the rat 5-HT_{4L} or the rat 5-HT_{4S} receptors. Cells were electroporated with 200 ng of each receptor cDNA/10^6 cells. In the absence of agonist the percentage conversions of [^{3}H]ATP to [^{3}H]cAMP in LLCPK1 without transfection or transfected either with rat 5-HT_{4S} or rat 5-HT_{4L} or mouse 5-HT_{4L} receptor cDNA were $0.11\pm0.05\%$, $0.12\pm0.03\%$, $0.14\pm0.06\%$ and $0.55\pm0.17\%$, respectively. In the presence of 100 μM 5-HT the conversions were $0.25\pm0.08\%$, $0.710\pm0.16\%$, $0.820\pm0.13\%$, and $1.23\pm0.11\%$, respectively. The results are expressed as the percentage stimulation of basal activity. B: Stimulation of cAMP formation by increasing concentrations of 5-HT in the absence or in the presence of 30, 100 or 300 nM of the highly selective 5-HT_4 antagonist: GR 113808, in LLCPK1 cells expressing mouse 5-HT_{4L} (200 ng of receptor cDNA/10^6 cells). In the absence of 5-HT, the basal percentage conversions of [^{3}H]ATP to [^{3}H]cAMP were $0.57\pm0.15\%$, $0.52\pm0.6\%$; $0.60\pm0.4\%$ and $0.51\pm0.7\%$, respectively, in the absence and in the presence of 30, 100 or 300 nM of GR 113808 100 μM. In the presence of 100 μM 5-HT, the maximal conversion obtained was $1.1\pm0.18\%$. Results are expressed as percentage of stimulation relative to the maximal stimulatory effect of 5-HT. In (A) and (B) each curve represents the mean from three separate experiments performed in duplicate.
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e1 and e2 likely to be engaged in a disulfide bridge, the DRY sequence at the N-terminus of the second intracellular loop i2 so important for activation of these receptors [17], the prolines in TMD-V, TMD-VI and TMD-VII and a cysteine (C328) which is a potential palmitoylation site [18,19]. Mouse 5-HT_{4L} receptor carries only two potential protein kinase C (PKC) phosphorylation sites (over three present in rat receptor). Amino acid sequence comparison of the C-terminus tail of mouse and rat 5-HT_{4L} receptors revealed a high level of identities, both are rich in serine and threonine residues (14 in mouse compared to 16 in rat). As previously demonstrated for the β_2-adrenergic receptor some of these residues are potential phosphorylation sites for G receptor protein kinase (GRK)s [20]. In the transmembrane domains, five differences within the amino-acid sequences of rat and mouse receptors were noted, including at position 75 in TMD-II, an alanine in rat which was occupied by a proline in the mouse sequence. In addition, a proline (P242) was present in the third intracellular loop (i3) of mouse 5-HT_{4L} receptor which was absent in rat 5-HT_{4L} receptor (T242).

3.2. Pharmacological characterization of mouse 5-HT_{4L} receptor

The cDNA encoding the mouse 5-HT_{4L} receptor was transiently transfected in LLCPK1 cells. No detectable binding was found in mocked transfected cells using ^{3}H-labeled GR 113808, as specific 5-HT_4 ligand (data not shown). The expression of receptors in LLCPK1 cell membranes increased with the amount of DNA transfected (Fig. 2A). Only a single category of binding sites was observed ($K_d = 0.22\pm0.07$ nM). In order to compare the pharmacological properties of the cloned mouse 5-HT_{4L} receptor with those of the 5-HT_4 receptor expressed in native colliculi neurons [11] we measured the affinities of 11 agonists and five antagonists using displacement experiments. All the displacement curves were monophasic, giving a Hill coefficient not different from 1. A remarkable correlation ($r = 0.978$) was found between the affinities of agonists and antagonists for 5-HT_4 receptor in colliculi neurons and for the cloned mouse 5-HT_{4L} receptor transfected in LLCPK1 (Fig. 2B). We also verified that the mouse 5-HT_{4L} receptor transiently expressed in LLCPK1 was positively coupled to AC. The EC_{50} of the 5-HT dose–concentration curves obtained in transfected cells with the mouse

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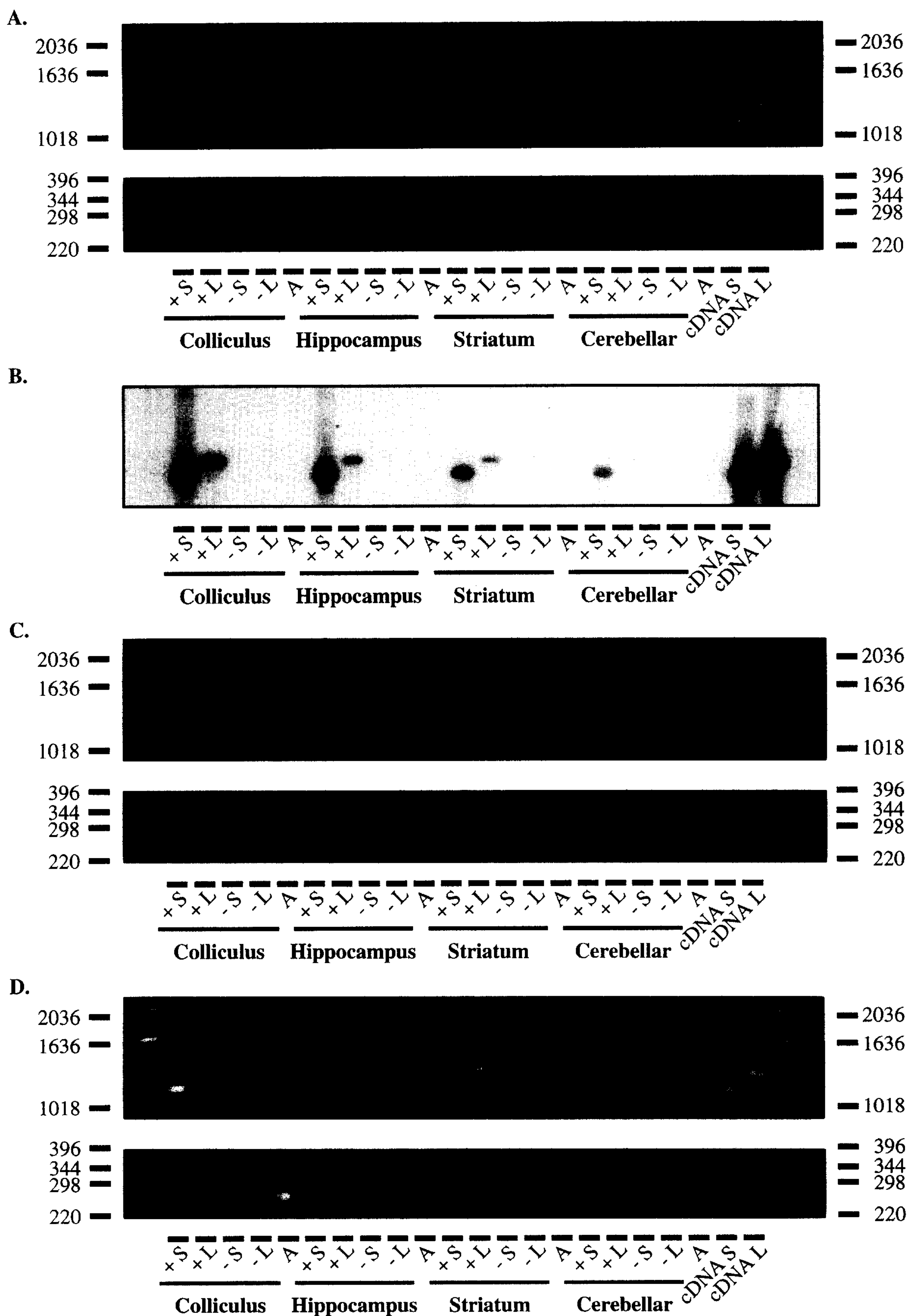
Fig. 4. Central distribution of 5-HT_{4S} and 5-HT_{4L} transcripts. RT-PCR analysis performed with 100 ng of poly(A^+) RNA from various structures of new born mouse brain (A,B), adult mouse brain (C) and adult rat brain (D). The PCR amplified products were resolved by 1% agarose/ethidium bromide gel electrophoresis, photographed and analysed by Southern blotting (see Section 2). Southern blot of new born mouse brain PCR results exposed 90 min to X-ray films (B). Specific primers based on the known rat cDNA sequences 5-HT_{4S}, 5-HT_{4L} or actin (see Section 2) were used on poly(A^+) RNA treated with (+S, +L, A) or without reverse transcriptase (−S, −L). A positive control was done by PCR reaction performed with rat cDNA clones encoding 5-HT_{4S} and 5-HT_{4L} receptors. The marker fragments indicated on the right and the left of the photograph show that the size of the PCR products (about 1300 bp) correspond to the total cDNA encoding 5-HT_{4S} and 5-HT_{4L} receptors.

and rat 5-HT_4 receptors cDNA (200 ng) were significantly different, 8 ± 3 nM (n = 6), 35 ± 12 nM (n = 4) and 24 ± 7 nM (n = 4) for mouse 5-HT_{4L}, rat 5-HT_{4L} and rat 5-HT_{4S}, respectively (Fig. 3A). Another clear difference was an increase in



basal AC level in cells transfected with mouse $5\text{-}HT_{4L}$ receptors compared to that transfected with rat $5\text{-}HT_4$ receptors (Fig. 3A). This increase in basal AC activity corresponded to an intrinsic activity of the mouse $5\text{-}HT_4$ receptor, i.e., a coupling in the absence of agonist, and not to an agonist stimulated form of the receptor since this basal activity was not decreased by the highly potent antagonist GR 113808 at a concentration of 300 nM (see legend to Fig. 3B) which competitively inhibits the 5-HT dose–response curve (K_i=0.8 nM$\pm$0.3) (Fig. 3B). This intrinsic activity of mouse $5\text{-}HT_4$ receptor not seen with rat $5\text{-}HT_4$ receptors was certainly due to a much higher expression of mouse $5\text{-}HT_4$ receptor in LLCPK1 cells. Indeed, for the same amount of transfected cDNA (200 ng of cDNA/10^6 cells) we obtained 8 pmol and 0.1 pmol of mouse and rat $5\text{-}HT_{4L}$ receptor expressed per mg of protein.

3.3. *Distribution of $5\text{-}HT_{4S}$ and $5\text{-}HT_{4L}$ receptors in new born and adult mouse brain as well as in adult rat brain*

Gerald et al. [16] described that $5\text{-}HT_{4L}$ receptors are widely distributed in rat brain whereas $5\text{-}HT_{4S}$ has a very specific and unique expression in rat striatum. In order to evaluate a possible differential splicing during development, we decided to verify if this also occurs in new born mouse brain. Using pairs of primers specific for each isoform (see Section 2), we amplified cDNA derived from mRNA coding for the two splice variants from different brain areas (Fig. 4A). The identification of two fragments (1150 and 1293 bp) corresponding to sequences of $5\text{-}HT_{4S}$ and $5\text{-}HT_{4L}$, respectively, was based on two criteria: (1) a positive control was done by PCR reaction performed with rat $5\text{-}HT_{4S}$ and $5\text{-}HT_{4L}$ cDNA (a generous gift from Dr. Christophe Gerald) (Fig. 4A), (2) Southern blotting and hybridization with a ^{32}P-labeled probe common to both splice variants (5′ region → position 1157 probe) under high stringency specifically labeled the same fragments (Fig. 4B). The hybridization signals were not due to the contaminating genomic DNA, since $5\text{-}HT_{4S}$ and $5\text{-}HT_{4L}$ primers failed to yield any PCR fragments in the minus reverse transcriptase controls (Fig. 4A, B). Since the presence of $5\text{-}HT_{4S}$ mRNA in all new born mouse brain areas was unexpected [16], we studied the distribution of the $5\text{-}HT_4$ receptor isoforms in both mouse and rat adult brains for comparison. Finally, both $5\text{-}HT_4$ receptor isoforms mRNA were present in all mouse and rat brain areas (Fig. 4C,D).

4. Discussion

We have isolated cDNA encoding the $5\text{-}HT_{4L}$ receptor by RT-PCR on poly$(A)^+$ RNA from mouse colliculi using specific primers designed from the sequence of the rat $5\text{-}HT_{4L}$ cDNA. As expected, the sequence homology with the rat $5\text{-}HT_{4L}$ isoform was very high. Only 16 residues were different between the rat and mouse sequences of $5\text{-}HT_{4L}$ receptor. Three of these differences deserve specific comments.

(1) The residue at position 75, in TMD II, is an alanine in rat and a proline in mouse $5\text{-}HT_4$ receptors. The importance of the TMD II, in binding of ligands to receptors for biogenic amines, is well documented [18,19]. The proline[75] found in mouse $5\text{-}HT_4$ receptors is part of a consensus sequence (LVMP) found in TMD II of all 5-HT receptors [21], except in rat $5\text{-}HT_4$ in which the corresponding sequence is LVNA [16]. This striking amino acid sequence conservation in all 5-HT receptors except in rat $5\text{-}HT_4$ receptor was very surprising. Therefore, we decided to sequence this region in both rat brain $5\text{-}HT_{4L}$ and $5\text{-}HT_{4S}$ cDNA obtained by RT-PCR with poly$(A)^+$ RNA prepared from total brain. Our sequence analysis revealed that indeed in rat $5\text{-}HT_4$ receptors, the sequence is also LVMP.

(2) The presence of a proline in the middle of the third intracellular loop (i3) (P242) of the mouse receptor, which is the position equivalent to a threonine in the rat receptor, may have a crucial role in coupling to G proteins or to other putative functions of the third loop, such as cellular targeting.

(3) The suppression of a putative PKC phosphorylation site in the mouse $5\text{-}HT_{4L}$ receptor compared to the rat $5\text{-}HT_{4L}$ receptor since position 400 in the C-terminus is occupied by a glycine and a serine, respectively. We have no information about a possible desensitization of $5\text{-}HT_4$ receptors via PKC. In contrast, we do know that $5\text{-}HT_4$ receptors in mouse colliculi neurons and rat oesophagus are rapidly desensitized via homologous desensitization [22,23]. The homologous desensitization is certainly related to the phosphorylation of the numerous serine and threonine residues present in C-terminus (14 in mouse, 16 in rat $5\text{-}HT_{4L}$), but also in the second and third intracellular loops [20,24]. The absence of cAMP-PKA-mediated desensitization of mouse $5\text{-}HT_4$ receptors [22,23] is certainly related to the absence of consensus sequence for phosphorylation by PKA in the mouse $5\text{-}HT_4$ receptor clone described here.

The pharmacological profile of the mouse $5\text{-}HT_{4L}$ receptor, cloned from colliculi neurons is identical to that of native mouse colliculi neurons when the affinity constants of both agonists and antagonists are considered. The mouse $5\text{-}HT_{4L}$ receptor clone was much more efficacious than the rat receptor clone in terms of receptor synthesis and expression. The reason for this difference in expression is unknown. It could be related to the non-coding sequences present in the 5′ and 3′ regions which are very short in the mouse $5\text{-}HT_{4L}$ cDNA clone compared to the rat $5\text{-}HT_{4L}$ and $5\text{-}HT_{4S}$ cDNA clones. The analysis of the untranslated regions reveals many initiator ATG followed by termination codons. These regions might affect translation efficiency and/or mRNA stability. These observations are in agreement with earlier reported data which demonstrates that removing the 5′ untranslated region containing several ATG codons separated by short reading frames and termination codons, results in a 10-fold increase in translation of the β_2AR mRNA [25]. In our case, absence of the 5′ untranslated region lead to an overexpression of mouse $5\text{-}HT_{4L}$ receptor which gives a constitutive coupling to Gs protein and constitutive activation of AC. Such a constitutive coupling has been described in three situations. (1) In some receptor families like the metabotropic glutamate receptor family, some spliced variants are spontaneously coupled to G proteins [26]. (2) In some receptors specific mutations (found in pathologies or experimentally done) give a constitutive activity [27]. (3) Overexpression of some receptors like the β_2-adrenergic receptors both in vitro and in vivo lead to a constitutive activation of Gs [28].

Finally, one of the most suprising findings of the present report was the fact that the two splice variants of $5\text{-}HT_4$ receptors ($5\text{-}HT_{4L}$ and $_S$) were expressed in all areas of adult mouse and rat brain studied as well as in new born mouse brain. This is in contrast to the report of Gerald et al. [16] who, using a similar PCR method, described a unique expres-

sion of 5-HT_{4S} in rat striatum. The reason for this discrepancy is unknown and deserves further investigation.

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