Chicken neuropeptide Y receptor Y2: structural and pharmacological differences to mammalian Y2[1]

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Abstract Here we report the molecular cloning of the chicken (*Gallus gallus*) neuropeptide Y (NPY) receptor Y2, the first non-mammalian Y2 receptor. It displays 75–80% identity to mammalian Y2 and has a surprisingly divergent cytoplasmic tail. Expression of the receptor protein in a cell line showed that the receptor did not bind the mammalian Y2 selective antagonist BIIE0246. Furthermore, porcine [Leu^{31}, Pro^{34}]NPY, which binds poorly to mammalian Y2, exhibited an unexpectedly high affinity for chicken Y2. In situ hybridisation revealed expression in the hippocampus. Thus, the chicken Y2 receptor exhibits substantial differences with regard to sequence and pharmacological profile in comparison to mammalian Y2 receptors, while the expression pattern in the central nervous system resembles that observed in mammals.

Key words: G-protein coupled receptor; Neuropeptide Y; Cloning; Expression; In situ hybridization; Chicken

1. Introduction

The neuropeptide Y (NPY) family of peptides consists in tetrapods of NPY, peptide YY (PYY) and pancreatic polypeptide (PP). NPY and PYY show a high degree of sequence identity and are found in all vertebrates [1]. The physiological effects of the peptides are mediated through receptors belonging to the G-protein coupled receptor superfamily. So far, five receptor subtypes have been cloned in mammals [2,3].

The Y1, Y2 and Y5 genes are localised on the same chromosome in human HSA4 [4,5] and pig SSC8 [6] suggesting, together with the low degree of identity, 30% at the amino acid level, that these subtypes resulted from ancient local duplications early in vertebrate evolution. Subtypes Y4 and y6 are found on human chromosome HSA10 and 5, respectively, and are approximately 50% identical to Y1 and each other. These genes presumably arose by duplication of the chromosome harbouring the Y1 gene [6], as well as Y2 and Y5 whose duplicates were probably lost. Indeed, the human genome has several segments that signify early chromosome or genome duplications [7]. Thus, Y1, Y4 and y6 form a subfamily within the NPY receptor family. The five subtypes differ extensively in their replacement rates as shown by comparisons across orders of mammals. Y1 and Y2 are the most highly conserved (each has 95% identity) whereas Y4 and y6 evolve quite rapidly (75–85% identity) [6]. Y5 is intermediate; however, its variability is mostly restricted to the large third cytoplasmic loop [6].

The physiological effects of the NPY family of peptides are diverse. The stimulation of appetite, regulation of circadian rhythm and blood pressure and inhibition of anxiety are among the most prominent [8,9]. Certain effects are believed to be mediated primarily by the Y2 receptor subtype, including regulation of circadian rhythms, inhibition of presynaptic transmitter release in the central nervous system as well as the peripheral nervous system, modification of the electrophysiological properties of hippocampal neurons, nasal congestion and gastro-intestinal and renal epithelial secretion [10–12].

NPY has been shown to bind to all Y receptors, albeit with low affinity to Y4. PYY binds to, and can mimic the actions of, NPY on all subtypes except Y3 [13], which has not yet been cloned. The mammalian Y2 receptors exhibit the same pharmacological profile binding both NPY and PYY and a number of truncated NPY analogues as well as the Y2 selective antagonist BIIE0246. None of the previously characterised Y2 receptors bind endogenous PP from rat (r) and human (h), or the modified NPY analogue porcine (p) [Leu^{31}, Pro^{34}]NPY, which have therefore been used to differentiate between the Y2 and other NPY receptor subtypes [14–16].

The complexity and diversity of the biological actions of the NPY family of peptides and their receptors is not fully understood. Characterisation of the receptors in more distantly related species may help resolve both the evolution of the receptor–peptide system and the primary physiological functions, as well as assist in generating further sequence information in order to clone and characterise receptor subtypes in other more primitive vertebrate species.

The present study describes a chicken (ch) clone encoding a Y2 receptor. We present here the sequence, anatomical mRNA distribution, as well as the pharmacological profile using a number of analogues, fragments and derivatives of NPY.

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[1] The nucleotide sequence reported in this paper has been submitted to the GenBank™/EMBL with the accession number: AF309091.

Abbreviations: NPY, neuropeptide Y; PYY, peptide YY; ch, chicken; h, human; p, porcine; r, rat

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2. Materials and methods

2.1. Screening of genomic library

Approximately 700 000 clones representing roughly four genome equivalents from a library in the vector EMBL3 were screened using two polymerase chain reaction (PCR) generated ^{32}P-labelled full length Y2 fragments from rat and human. Hybridisation was carried out at 42°C in 25% formamide, 6×SSC, 10% dextran sulphate, 5×Denhardt's solution and 0.1% SDS over night. The filters were washed twice in 2×SSC/0.1% SDS at room temperature for 5 min, and twice in 0.5×SSC/0.1% SDS for 30 min at 42°C. One clone hybridising to two duplicate filters was selected and digested using *Eco*RI. Southern blot and subsequent hybridisation with the human and rat Y2 probes revealed two bands with sequence homology to the probe fragments. A 2.1 kb fragment containing 800 bp of the chY2 gene was cloned in pBluescript KS+vector and sequenced. DNA preparations from the selected phage clone were sequenced using specific sequencing probes designed from the cloned fragment. Primers were then designed in order to obtain a full length sequence.

2.2. DNA sequencing and amino acid alignment

Sequence determinations were performed using ABI PRISM Dye Terminator cycle sequencing kit according to the manufacturer's directions (Perkin Elmer) and analysed by an automated ABI-310 fluorescent-dye sequencer (Applied Biosystems). Sequence alignments were performed using Lasergene DNASTAR Megalign software. Transmembrane regions were based upon Schwartz et al. [17]. A sequence distance tree was made using alignments with Y2, Y1 and Y5 sequences and, as an outgroup, bradykinin B1 and B2 receptor amino acid sequences retrieved from GenBank.

2.3. Cloning in expression vector

A fragment containing the entire coding region of the chY2 gene was generated with Platinum Pfx DNA polymerase (Gibco BRL), using specific primers containing *Eco*RI and *Bam*HI sites. Cycle: 20 s at 95°C, 30 s at 50°C and 1 min 20 s at 68°C for 35 cycles. The PCR fragment was purified by Qiagen PCR product purification kit (Qiagen), digested using *Eco*RI and *Bam*HI. The 1.2 kb fragment was then purified with QIAquick PCR purification kit (Qiagen) and then ligated into a pTEJ8 expression vector to give the clone chY2-pTEJ8. The construct was sequenced and found to be identical to the genomic clone.

2.4. Transfection protocol

For transient transfections HEK293 (EBNA) cells were transfected with the construct chY2-pTEJ8 using FuGENE™ Transfection Reagent (Boehringer Mannheim, Germany), diluted in OptiMEM medium (Gibco BRL, Stockholm, Sweden) according to the manufacturer's recommendations. After transfection, cells were grown in Dulbecco's modified Eagle's medium (DMEM)/Nut Mix F-12 w/o L-glutamine (Gibco BRL) containing 10% foetal calf serum (Biotech Line AS, CA, USA), 24 mM L-glutamine (Gibco BRL) and 250 μg/ml G-418 (Gibco BRL), penicillin–streptomycin (100 U penicillin, 100 μg streptomycin/ml) (Gibco BRL) until harvesting by centrifugation, after 48 h. Cell membrane pellets were frozen in aliquots at −80°C. To obtain semi-stable cells for studies of cyclic AMP (cAMP), HEK293 (EBNA) cells were grown in DMEM containing 10% foetal calf serum and 250 μg/ml G-418. Cells were transfected with 6 μl FuGENE™ and 2 μg of the construct chY2FLAG-pCEP4 (which contains the nine amino acid FLAG epitope in the very carboxy terminus). After 24 h, the cells were split and 400 μg/ml hygromycin B (Gibco BRL) was added to the medium. A control plate with untransfected cells was treated the same way and after 7 days all cells in the control plate were dead while the transfected plate was confluent. The cells from the transfected plate were grown under hygromycin selection and subsequently checked for ^{125}I-pPYY binding. The pharmacology of the stably expressed chY2 receptor was indistinguishable from that of the transiently expressed receptor reported in this paper.

2.5. Peptides and peptidic and non-peptidic antagonists

Porcine NPY, p[Leu^{31}, Pro^{34}]NPY, pNPY2–36, pNPY3–36, pNPY18–36, hPP and rPP were purchased from Bachem, King of Prussia, PA, USA; p[D-Trp^{32}]NPY from Peninsula Laboratories, CA, USA. BIBP3226 [18] was synthesised at Dr. Karl Thomae's laboratories. SR120819A [19] was provided by Sanofi, chicken PP and PYY were purchased from Schafer-N, Copenhagen, Denmark. Non-peptidic Y2 antagonist BIIE0246 [20] was provided by Boehringer-Ingelheim PharmaKG, Biberach an der Riss, Germany.

2.6. Binding assays

The thawed aliquots of membranes were resuspended in 25 mM HEPES-buffer (pH 7.4) containing 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 2 g/l bacitracin and homogenised using an Ultra-Turrax homogeniser. Saturation experiments were performed in a final volume of 100 μl with 4–5 μg protein and ^{125}I-pPYY (Amersham, UK) for 2 h at room temperature. This radioligand is iodinated at tyrosines 21 and 27 and has a specific activity of 4000 Ci/mmol. Saturation experiments were carried out with serial dilutions of radioligand. Non-specific binding was defined as the amount of radioactivity remaining bound to the cell homogenate after incubation in the presence of 100 nM unlabelled pNPY. Competition experiments were performed in a final volume of 100 μl. Various concentrations of the peptides pNPY, hPP, rPP, chPP, chPYY, p[Leu^{31}, Pro^{34}]NPY, pNPY2–36, pNPY3–36, pNPY13–36, pNPY18–36, p[D-Trp^{32}]NPY, two non-peptidic Y1 antagonists SR120819A and BIBP3226, and one non-peptidic Y2 antagonist BIIE0246 were included in the incubation mixture along with ^{125}I-pPYY. Incubations were terminated by filtration through GF/C filters, Filtermat A (Wallac Oy, Turku, Finland), that had been presoaked in 0.3% polyethyleneimine, using a TOMTEC (Orange, CT, USA) cell harvester. The filters were washed with 5 ml of 50 mM Tris (pH 7.4) at 4°C and dried at 60°C. The dried filters were treated with MeltiLex A (Wallac) melt-on scintillator sheets and the radioactivity retained on the filters counted using the Wallac 1450 Microbeta counter. The results were analysed using the Prism 2.0 software package (Graphpad, San Diego, CA, USA). Protein concentrations were measured using a Bio-Rad Protein Assay (Bio-Rad, Solna, Sweden) with bovine serum albumin as standard.

2.7. Inhibition of forskolin stimulated cAMP synthesis

Cyclic AMP was assayed on stably transfected HEK293 (EBNA) cells. The cells were detached by pipetting with media, diluted to 1000 cells/μl and treated for 30 min at 37°C with 250 μM isobutylmethylxanthine (Sigma). About 200 000 cells (200 μl) were incubated with 10 μM forskolin (Sigma) and various concentrations of chPYY, pNPY, p[Leu^{31}, Pro^{34}]NPY, pNPY2–36, or pNPY18–36 for 20 min at 37°C in a total volume of 250 μl. The ability of BIIE0246 to inhibit the effect of 100 nM pNPY was also tested. Reactions were terminated by adding 25 μl $HClO_4$ (4.4 M) and the suspension was neutralised by adding 40 μl KOH (5 M). Membranes were pelleted by centrifugation and 50 μl of the supernatant was used to quantify cAMP with a radioassay using ^{3}H-cAMP (Amersham Pharmacia Biotech) as a competitor. After 2 h, bound ^{3}H-cAMP was separated from free by the addition of 150 μl of a 1% charcoal suspension (50 mM Tris, 4 mM EDTA). The tubes were vortexed and spun at maximum speed for 2 min, 300 μl of the supernatant was rapidly removed and counted in a liquid scintillation analyser (Packard). Cyclic AMP binding protein was extracted from the bovine adrenal cortex.

2.8. In situ hybridisation

Brains from four Bantam chickens (two male, two female; Roslin Institute flock) were processed for in situ hybridisation. The birds were maintained on a photoperiod of 14L10D and had free access to food and water. Brains were rapidly dissected from birds killed by cervical dislocation and were immediately frozen in powdered dry ice. The tissue was stored at −70°C before being sectioned on a cryostat (Shandon, Model OT) at 15 μm thickness. Coronal sections were thaw-mounted onto microscope slides (Superfrost Plus, Cellpath, Hemel Hempstead, Herts, UK) and stored at −70°C. Slides bearing brain sections were processed by sequential immersion at room temperature in: 4% paraformaldehyde (5 min); twice in 0.1 M sodium phosphate (5 min each); water (< 5 s); 0.1 M triethanolamine, pH 8.0 (TEA, < 5 s); 0.15 M acetic anhydride in 0.1 M TEA (10 min); 2×SSC; 70, 95 and 100% ethanol (3 min each); and blow-dried. Slides were then stored at room temperature until application of the hybridisation solution. A mixture of two oligonucleotide probes complementary to chicken Y2 receptor mRNA was used for in situ hybridisation. The oligonucleotide sequences were: 5′CGATCACCCCCAACAGAATAATGGAACAGTAAGCAAAGATGAGGAT-3′ and 5′CTGATACGCTTAGAGATTTTGCTTTCCAAGTGATAGACAATACCACGAT-3′. The probes were labelled at the 3′ end with [^{35}S]dATP using terminal deoxynucleotidyl transferase (Amersham,

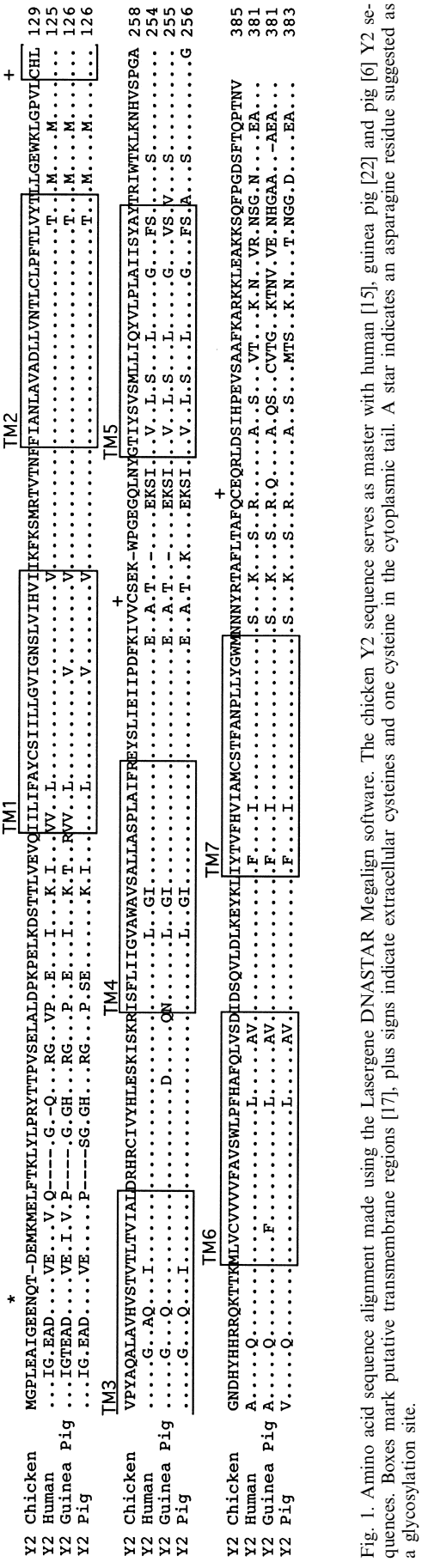

Fig. 1. Amino acid sequence alignment made using the Lasergene DNASTAR Megalign software. The chicken Y2 sequence serves as master with human [15], guinea pig [22] and pig [6] Y2 sequences. Boxes mark putative transmembrane regions [17], plus signs indicate extracellular cysteines and one cysteine in the cytoplasmic tail. A star indicates an asparagine residue suggested as a glycosylation site.

Little Chalfont, Bucks, UK) and were purified through QIAquick spin columns (Qiagen, Crawley, West Sussex, UK). Specific activity of the probes was 10^8 cpm/μg oligonucleotide. Hybridisation buffer consisted of 50% formamide, 4×SSC, 10% dextran sulphate, 1% *N*-laurylsarcosine, 1×Denhardt's solution, 200 mM dithiothreitol, and 0.1 mg/ml yeast tRNA. A 100 μl aliquot of hybridisation solution containing 3 ng of each labelled probe was applied to each section and covered with a Parafilm coverslip. Control sections received hybridisation solution containing a 100-fold excess of each unlabelled probe. Hybridisation was performed for at least 16 h in humidified boxes at 42°C. After hybridisation, the sections were washed (4×45 min) in 1×SSC at 56°C, and brought to room temperature while in the final wash. The tissue was then dehydrated through graded alcohols and apposed to Kodak Biomax MR film for 2–3 weeks at room temperature. Films were developed using an automatic processor (X-ograph Imaging Systems, Tetbury, Gloucestershire, UK).

3. Results

A chicken genomic phage library was screened using two full length Y2 PCR products, human Y2 and rat Y2, under low stringency conditions. A strongly hybridising clone was isolated and an 800 bp fragment isolated from the phage was subcloned in order to obtain a partial length chicken Y2 product. The fragment was sequenced and used to design primers with which the phage clone was sequenced by sequence primer walking.

The open reading frame encodes a protein of 385 amino acid residues which displays the characteristic features of a G-protein coupled receptor, i.e. seven putative transmembrane regions, a cysteine pair linking extracellular loop 1 and loop 2, and a cysteine in the carboxy terminal tail where palmitoylation could serve as an anchor to the membrane and form a pseudo fourth loop (Fig. 1). The receptor also contains an asparagine residue close to the amino terminal, which has been identified as a putative glycosylation site in mammalian Y2 receptors. The protein has 78–79% identity to the human, murine, porcine and bovine Y2 receptors and 75% identity to the guinea pig Y2 subtypes. The mammalian subtypes share 90–94% identity between each other. When compared to other NPY receptor subtypes the chicken Y2 receptor exhibits con-

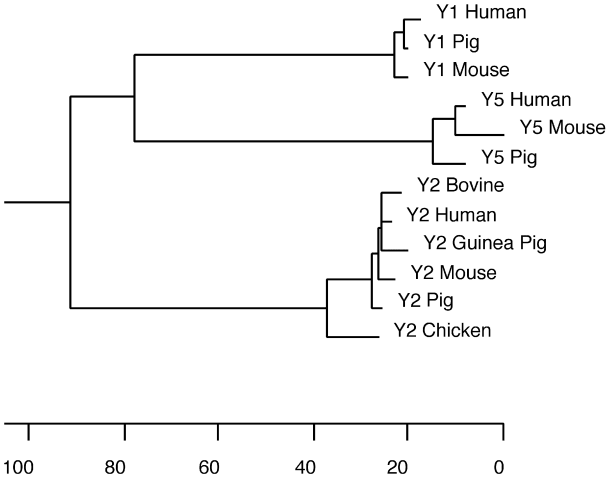

Fig. 2. Distance tree for the characterised NPY Y2 receptors, three Y5 and three Y1 receptors. Branch lengths correspond to sequence divergence using the neighbour joining method of the Lasergene DNASTAR Megalign software. The human bradykinin B1 and B2 receptors were used as outgroup to root the tree.

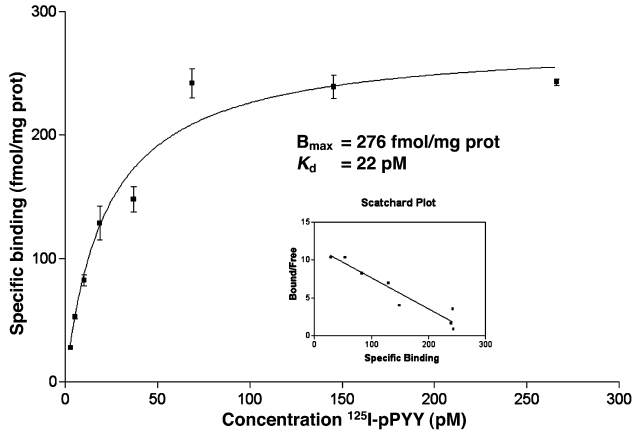

Fig. 3. Saturation binding isotherm and Scatchard (inset) analyses of ^{125}I-pPYY binding to membranes prepared from HEK293 (EBNA) cells transfected with the chY2 expression plasmid chY2-pTEJ8. The figure shows results from a representative experiment.

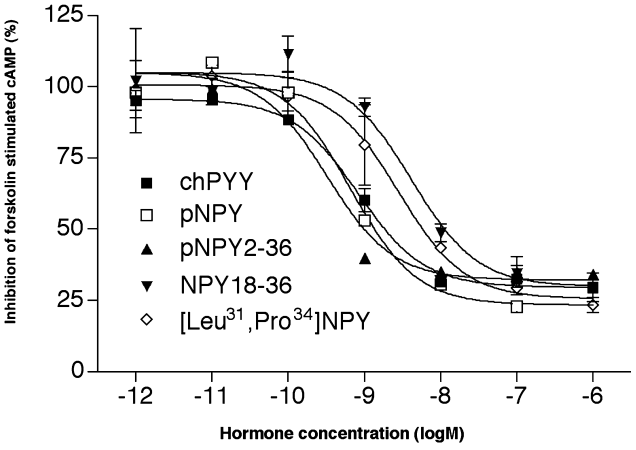

Fig. 4. Inhibition of forskolin stimulated cAMP synthesis. Baseline represents unstimulated cells and 100% represents cells after stimulation of 10 μM forskolin. The figure shows one representative experiment.

siderably less identity: 23–26% to Y1, Y4, Y5 and y6 subtypes in mammals (Fig. 2). The alignment reveals that divergence to mammalian Y2 has primarily occurred in the carboxy and amino terminals while the receptor is more highly conserved in the transmembrane and loop domains. The genomic clone described here was found to be intronless in the coding region as is the case for the Y2 genes in mammals.

The coding sequence of the genomic clone was transferred to the expression vector pTEJ8 [21] and control sequenced. The chY2-pTEJ8 construct was transfected into HEK293 (EBNA) cells and membranes prepared from the transfected cells were then used for radioligand binding assays. The membrane fraction exhibited concentration dependent binding of ^{125}I-pPYY with an affinity constant (K_d) of 25.6 pM (S.E.M. = 1.7, $N = 3$) and a B_{max} of 303 fmol/mg protein (S.E.M. = 68, $N = 3$) (Fig. 3). All tested NPY variants, pNPY and chPYY were potent inhibitors of ^{125}I-pPYY. The inhibition constants (K_i) are presented in Table 1. Weak inhibition was seen with p[D-Trp^{32}]NPY ($K_i = 400$ nM) and no inhibition was detected with the Y1 antagonists BIBP3226 and SR120819A, rPP, hPP or the Y2 antagonist BIIE0246 ($K_i > 1$ μM). Functional coupling to G-proteins was shown by inhibition of cAMP synthesis. Chicken PYY ($pEC_{50} = 9.12 \pm 0.09$, $n = 6$), pNPY (9.02 ± 0.18, $n = 5$), and pNPY2–36 (8.95 ± 0.28, $n = 3$) were equally potent while NPY18–36 (8.27 ± 0.40, $n = 5$) and p[Leu^{31}, Pro^{34}]NPY (8.27 ± 0.21, $n = 5$) were about four-fold less potent (Fig. 4). The Y2 antagonist BIIE0246 up to a concentration of 1 μM did not affect the inhibition from 100 nM pNPY (data not shown).

Table 1
Inhibition of ^{125}I-pPYY binding to membranes from HEK293 (EBNA) cells transfected with the chY2-pTEJ8 expression construct using NPY, truncated NPY peptides and non-peptide analogues

Ligand	pK_I ($-\log M$)	S.E.M.	n
chPYY	9.20	0.08	3
pNPY	9.46	0.06	3
pNPY2–36	9.26	0.06	3
pNPY3–36	9.13	0.06	3
pNPY13–36	8.91	0.08	3
pNPY18–36	7.70	0.10	3
[Leu^{31}, Pro^{34}]NPY	8.25	0.05	3
rPP	< 6.0		3
hPP	< 6.0		3
chPP	< 6.0		3
BIBP3226	< 6.0		3
SR120819A	< 6.0		3
p[D-Trp^{32}]NPY	6.42	0.11	3
BIIE0246	6.20	0.06	3

In situ hybridisation with oligonucleotide probes to the chicken Y2 receptor revealed a prominent signal in the hippocampus (Fig. 5a). No signal was observed in this region after competition with excess unlabelled probe (Fig. 5b).

4. Discussion

NPY receptors previously cloned in species from several orders of mammals have revealed that the rates of evolution differ considerably between subtypes [6]. To obtain a greater understanding of the evolution of the NPY receptor gene family, and the function of the Y2 subtype in particular, we have undertaken the molecular cloning of a non-mammalian Y2 receptor. The chicken Y2 receptor described in this paper exhibits 75–80% identity with mammalian Y2 sequences, which is in agreement with the 90–95% identity between mammals and further supports that it is a well conserved receptor (Fig. 1).

A higher degree of identity was seen within the postulated transmembrane regions, whereas some divergence can be seen in the loop regions, and particularly in the amino and carboxy termini. In addition to the DRH motif located at the carboxy terminal end of the third transmembrane region typical for NPY Y2 subtypes, the amino acid translation reveals conserved postulated structurally important amino acids for disulphide bridges, palmitoylation and glycosylation [1]. A phylogenetic tree with other NPY receptor sequences places the chicken Y2 sequence closest to the mammalian Y2 sequences, and at a substantial distance from the other receptor subtypes (Fig. 2).

Cloning into a eukaryotic expression vector allowed us to express the receptor gene in a mammalian cell line. A wide variety of peptides, truncated peptides and analogues were used to determine the pharmacological profile in competition with iodinated pPYY. The properties proved to be deviant

from previously characterised Y2 receptors [15,16,22–24], with the following binding in rank order of potency: pNPY = chPYY = pNPY2–36 = pNPY3–36 > pNPY13–36 > pNPY18–36 = p[Leu^{31}, Pro^{34}]NPY≫p[D-Trp^{32}]NPY (Table 1). No binding ($K_i > 1$ μM) was observed for BIBP3226, SR120819A, rPP, hPP and BIIE0246. Two of the tested ligands displayed binding affinities that differ considerably from known interactions with mammalian Y2 receptors: BIIE0246, a potent antagonist at the human [20], rat [25] and guinea pig [26] Y2 receptors, did not exhibit specific binding at the chY2 receptor; and p[Leu^{31}, Pro^{34}]NPY that does not bind to previously characterised Y2 receptors, e.g. $K_i < 1$ μM, binds to the chY2 receptor with relatively high affinity. Functional coupling to G-proteins was also studied. Chicken PYY, pNPY and pNPY2–36 inhibited cAMP synthesis in stably transfected HEK293 (EBNA) cells with EC_{50} values of about 1 nM (Fig. 4). The truncated peptide pNPY18–36 displayed an EC_{50} of 5 nM, i.e. four times higher than the K_i value. Interestingly, p[Leu^{31}, Pro^{34}]NPY was also a full agonist only fivefold less potent than chPYY. BIIE0246 did not inhibit the effect of 100 nM pNPY in agreement with the competition studies. The similarity of chY2 to mammalian Y2 in the ability to inhibit cAMP synthesis indicates that the divergent carboxy terminal tail does not alter the interaction with G_i proteins.

The p[Leu^{31}, Pro^{34}]NPY peptide does not bind to any of the known mammalian Y2 receptors. It was designed to be more similar to the mammalian PP [27] that primarily binds to the NPY Y4 subtype [28]. In p[Leu^{31}, Pro^{34}]NPY the Glu^{34} residue of mammalian NPY has been replaced by a proline residue found at the corresponding position of all mammalian PP sequences. This gives the peptide a higher affinity for the mammalian Y4 subtype, i.e. the PP receptor [28]. Chicken PP, as well as other known avian PP sequences, differs from the mammalian PP peptides by having a histidine at position 34 [1]. This difference may explain why the avian Y2 receptor would not have the need to discriminate against the Pro^{34} residue in the p[Leu^{31}, Pro^{34}]NPY analogue. This reveals an important difference between the avian and mammalian NPY peptide–receptor interactions, which should prove useful for mutagenesis studies.

A molecular explanation for the lack of binding of the BIIE0246 antagonist cannot yet be given, but considering the evolutionary distance between the mammalian and avian lineages, and the fact that BIIE0246 was designed to bind to the human and rat Y2 receptors [20] this is not unexpected.

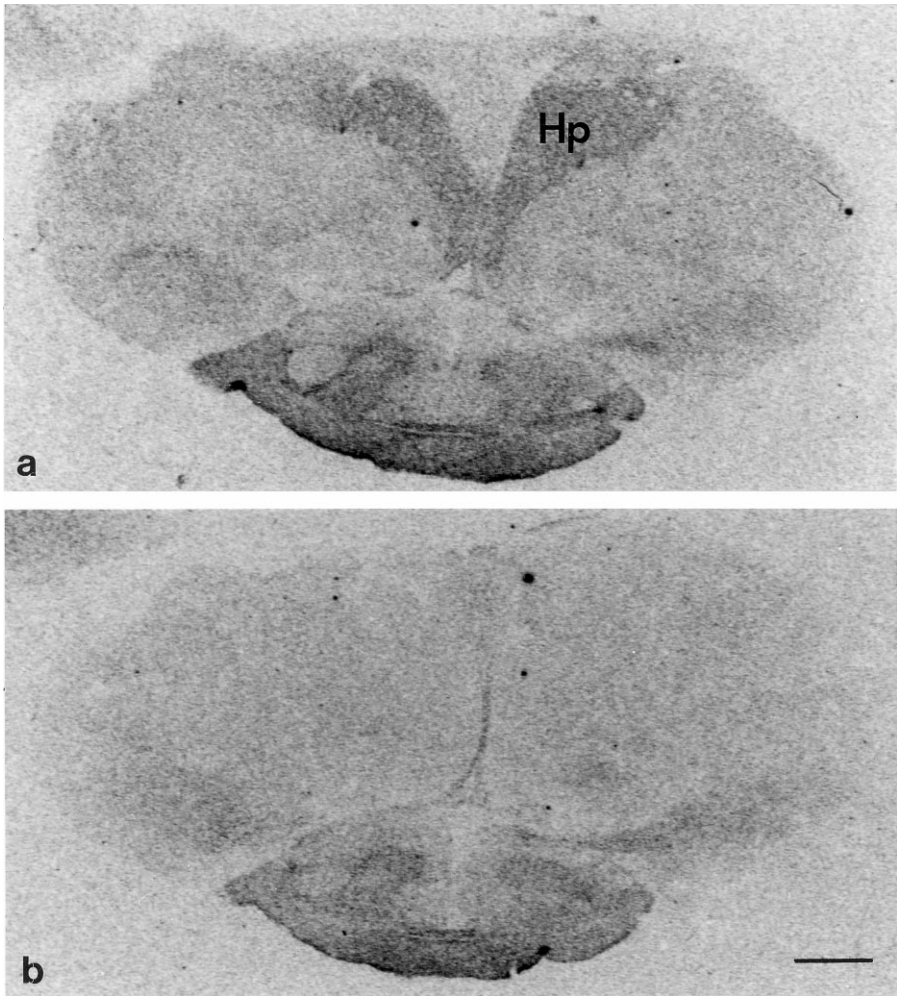

Fig. 5. In situ hybridisation of ^{35}S end-labelled oligonucleotides to chicken NPY Y2 receptor mRNA in chicken brain sections. A specific hybridisation signal in the hippocampus (Hp) (a) disappears in an adjacent section after competition with an unlabelled oligonucleotide probe (b) while non-specific hybridisation to the optic chiasm and neural tracts is retained. Scale bar = 2 mm.

Unfortunately, this means that BIIE0246 cannot be used in functional studies in chicken. The sequence of the chY2 receptor may prove to be of great value when investigating binding sites for BIIE0246 at the human Y2 receptor by site-directed mutagenesis, and such experiments are in progress.

Although the amino acid sequence and pharmacological properties of the chicken Y2 receptor differ significantly from its mammalian counterparts, the localisation of the receptor to the hippocampus in the present study (Fig. 5) indicates that the neuroanatomical distribution is conserved between birds and mammals. Expression of the Y2 receptor in the hippocampus is consistently reported in mammalian species including rat [29,30], sheep [31] and human [32]. The expression of NPY receptors in the chicken hippocampus is not unexpected because this region displays high specific binding of radiolabelled NPY [33]. Furthermore, NPY mRNA and peptide have been co-localised in the hippocampus of chickens and Japanese quail [34]. Collectively, these findings suggest that the functions of NPY in this brain region are likely to have been conserved during evolution. The regulatory effects of NPY in the chicken hippocampus have not been elucidated. However, in mammals, interaction of NPY with the Y2 receptor in the hippocampus has been implicated in seizure modulation and in the facilitation of learning and memory [35]. It is noteworthy that the pattern of Y2 receptor expression within the hippocampus appears to differ between birds and mammals. Thus, in the present study, Y2 receptor mRNA was evenly distributed over the hippocampal region whereas, in the rat, expression is limited to specific sub-regions, particularly CA2 and CA3 pyramidal cells and granule cells [29,36]. The significance of this is unclear. However, a difference in the distribution pattern is predictable given that the neuroanatomical organisation of the hippocampus differs markedly between birds and mammals to the extent that subdivisions applied to the mammalian structure are not recognisable in birds [37].

In conclusion, we have cloned and characterised a chicken NPY Y2 receptor and found considerable differences in amino acid sequence as well as pharmacological binding profile. The receptor appears to have a similar pattern of expression in the brain as mammalian Y2. The sequence information obtained from the chicken Y2 receptor should be of great importance for achieving a greater understanding of the interactions between NPY as well as NPY-like ligands at the Y2 receptors.

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