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Cloning and functional expression of a brain G-protein-coupled ATP receptor

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A cDNA encoding a novel member of the G-protein-coupled receptor (GCR) superfamily, an ATP receptor, has been isolated from an embryonic chick whole brain cDNA library by hybridization screening. The encoded protein has a sequence of 362 amino acids (41 kDa) and shares no more than 27% amino acid identity with any known GCR. When expressed as a complementary RNA (cRNA) in *Xenopus* oocytes a slowly-developing inward current was observed in response to application of ATP. The pharmacology of this expressed protein defines it as a P_{2Y} purinoceptor.

G-protein-coupled receptor; ATP; P_2 purinoceptor; P_{2Y} subtype; Chicken brain

1. INTRODUCTION

Evidence has accrued for many years that ATP can act as a neurotransmitter or modulator in both the peripheral and the central nervous system [1–3]. The effects of extracellular ATP are mediated via specific receptors, the P_2 purinoceptors: which are entirely distinct from those that bind adenosine [2]. At least five subtypes of P_2 purinoceptors have been proposed pharmacologically [2,4], with P_{2X} and P_{2Y} purinoceptors the best described. However, none has as yet been characterised at the molecular level. The P_{2X} purinoceptor has the properties of an ATP-gated cation channel [5], whereas other P_2 purinoceptor subtypes appear to be members of the GCR superfamily [4,6].

At around the time of hatching, the brain of the chick is known to be in a phase of highly active expression of many receptor mRNAs, as confirmed in oocyte expression studies [7,8]. Investigating sources of ATP receptors, we found that this brain is, in fact, exceptionally rich in high-affinity ATP-binding sites of the P_{2Y} subtypes (J.S. and T.E.W., unpublished). We have cloned a brain partial cDNA [9] that encodes the guinea pig equivalent of an unidentified receptor [10] of the G-protein-coupled class, RDC1, which is most related in sequence to canine adenosine receptors [10]. To isolate related sequences, we used this clone to screen an embryonic chick whole brain cDNA library. In this report we describe the cloning and expression of a cDNA encoding a novel GCR that binds ATP. This receptor displays a P_{2Y} purinoceptor pharmacology distinct from the classical [2,4] type. On the basis of this pharmacological evidence, we designate this novel subtype as a P_{2Y1} purinoceptor. This designation is supported by the restricted expression of the corresponding mRNA transcript.

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2. MATERIALS AND METHODS

2.1. *Polymerase chain reaction, cDNA library screening and DNA sequencing*

Two degenerate oligonucleotide primers, from transmembrane domains II and VI of G-protein-coupled receptors, 5′-TAGGTC GAC(G/C)(G/C)TGT(G/C)(T/C)CTGGC(C/T)GTGGC(C/T)GC-(A/C)T-3′ and 5′-AGGACGAATTCGGG(G/A/C)(G/A)ICCAAGT CAG(A/G)AT(G/A)AAG(G/A)C-3′, were used to amplify other members of the GCR superfamily from guinea pig brain first-strand cDNA. The amplification conditions were as follows: 94°C, 1 min, 55°C, 1 min, 72°C, 1 min; 30 cycles [9]. Amplification products were subcloned, making use of the restriction endonuclease sites incorporated into the 5′-ends of each primer, into complementary restricted M13mp18 for sequence analysis (Sanger dideoxy chain termination, Sequenase kit, USB)

A 497-bp cDNA fragment that encoded part of a RDC1-like sequence [9] was labelled (random primer method) to a specific activity of approximately 1×10^9 dpm/μg and used to screen 5×10^5 recombinants of an embryonic chick whole brain cDNA library (λgt10, gift of A. Hicks [11]). Hybridisation was in 6 × SSC at 65°C for 18 h; the final wash conditions were 1 × SSC at 55°C. Three hybridization classes of strong to weak intensity were distinguished DNA from a

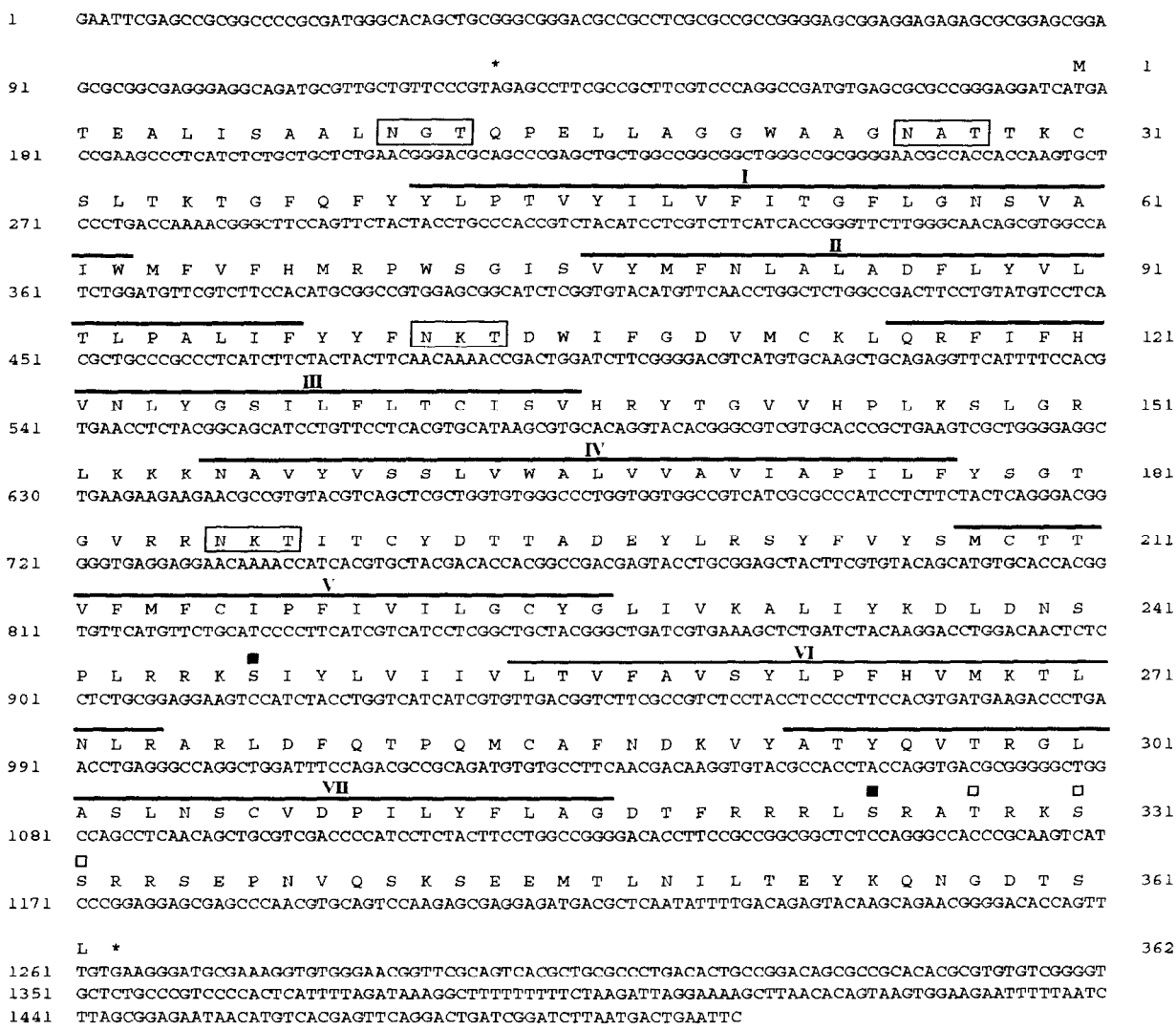


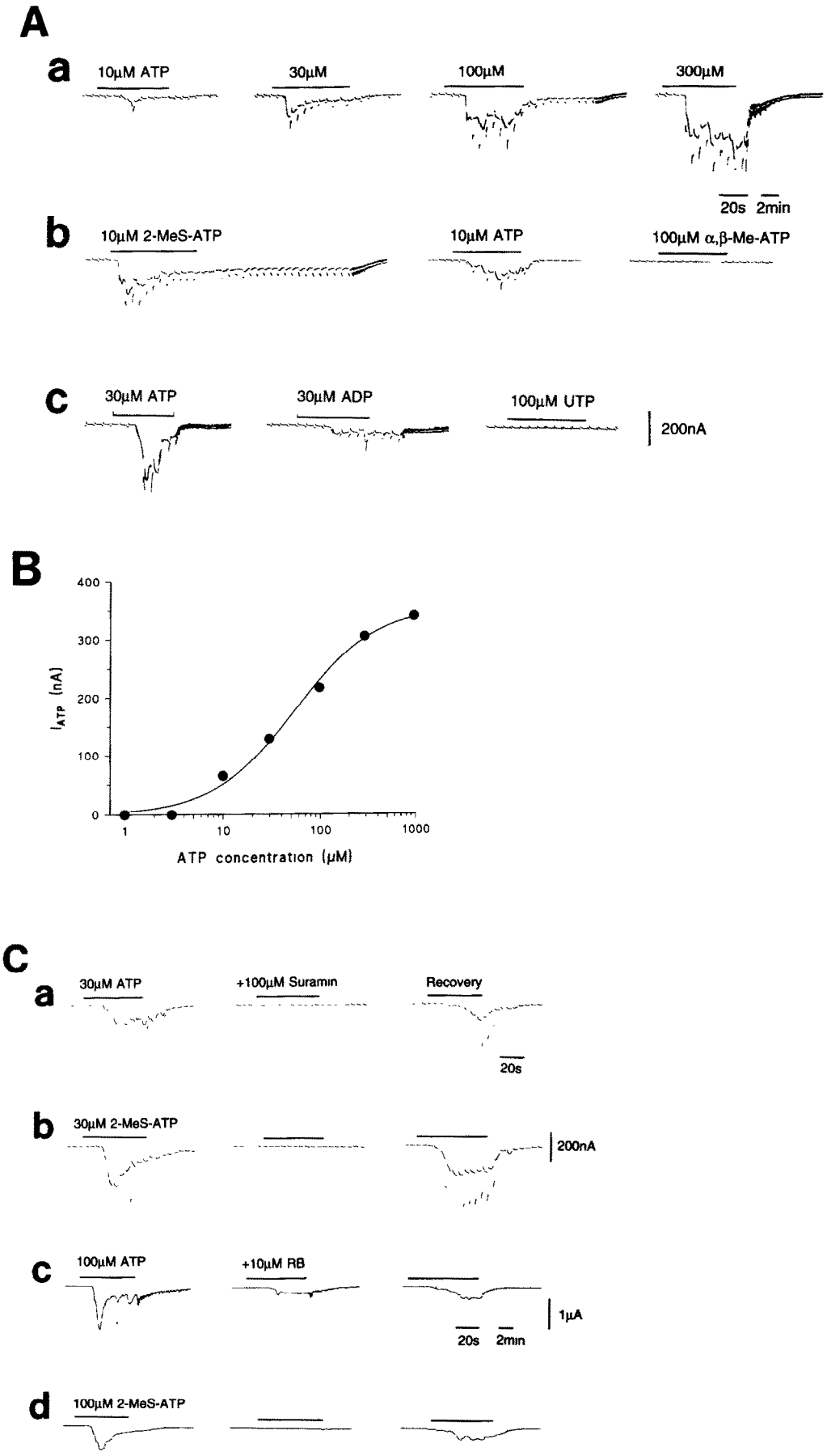
Fig. 1. Nucleotide and predicted amino acid sequence (single letter code) of the 803 protein. Putative transmembrane domains as determined by the Kyte–Doolittle algorithm [15] are overlined and numbered. Potential sites for N-linked glycosylation are boxed. Potential sites for phosphorylation by protein kinase A and protein kinase C are indicated by solid and open squares, respectively.

phage of middle intensity, clone 803, was isolated by standard procedures and the whole *Eco*RI insert was subcloned to the same site in M13mp18 and subjected to sequence analysis.

2.2. *In vitro transcription, oocyte preparation, microinjection and electrophysiological measurements*

The 1.5-kb cDNA insert from 803, was subcloned into the *Eco*RI site of the expression vector pSG5 (Stratagene). Capped and polyadenylated cRNA was transcribed in vitro from this construct, linearised with *Bam*HI, using a riboprobe kit (Promega). cRNA (~ 50 ng in 50 nl per oocyte) was injected into oocytes retaining their follicular envelope, as previously described [8]. After 2–3 days at 18°C in modified Barth's medium, they were maintained at 4°C for at least 11 days [8]. Two-electrode voltage clamping (Axoclamp 2A amplifier) was used at room temperature. The voltage-recording and current-injecting electrodes were filled with 0.6 M K_2SO_4 and 3 M KCl, respectively. Oocytes were superfused at a rate of 10 ml/min (bath volume 0.2 ml) with an amphibian Ringer (mM), 110 NaCl, 2 KCl, 5 HEPES, 2

Fig. 2. Responses in cRNA-injected *Xenopus* oocytes (at −40 mV). (Aa) Dose-dependence of membrane currents evoked by ATP (10–300 μM). The transient downward deflections monitor the input conductance following hyperpolarizing voltage steps (−10 mV, applied every 5 s for 1 s). Similar results were observed in two other oocytes. (Ab,c) In two oocytes, the agonist selectivity was assessed using 2-MeSATP, ATP, ADP, α,β-MeATP and UTP. Holding potentials, −40 mV. (B) ATP concentration–response relationship. The membrane current amplitude (I_{ATP}) was measured using the initial peak inward current induced by each ATP concentration. (C) Suramin antagonized the responses to (Ca) ATP and (Cb) 2-MeSATP. RB also inhibited the responses to (Cc) ATP and (Cd) 2-MeSATP. Note, in (A) and (C), the slower chart speed in some records during drug washout and the incomplete recoveries following treatment with RB. Bar indicates ligand application.


A
a
10μM ATP
30μM
100μM
300μM
20s 2min
b
10μM 2-MeS-ATP
10μM ATP
100μM α,β-Me-ATP
c
30μM ATP
30μM ADP
100μM UTP
200nA
B
400
300
200
100
0
I_{ATP} (nA)
1
10
100
1000
ATP concentration (μM)
C
a
30μM ATP
+100μM Suramin
Recovery
20s
b
30μM 2-MeS-ATP
200nA
c
100μM ATP
+10μM RB
1μA
20s 2min
d
100μM 2-MeS-ATP

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P2Y1                                MTEALISAALNGTQPELLAGGWAAGNATTKCS-LTKTGFQFY-----YLPTV  46
RDC1                             MDLHLFDYAEPGNFSDISWPCNSSDCIVVDTVLCPNMPNKSVLLY-----TLSFI  50
ANG II                                    MILNSSTEDGIKRIQDDCPKAGRHNYIFV-----MIPTL  34
Thrombin    MGPRRLLLVAACFSLCGPL-(54)-INKSSPLQKQLPAFISEDASGYLTSSWLTLFVPSV  108
PAF                                                 MELNSSSRVDSEFRY---TLF-PIV  21
A1                                                        MPPAISAFQAAYIG-I  15
A2                                                        MS-TMGSW--VYIT-V  12

P2Y1        YILVFITGFLGNSVAIWMF--VFHMRPWSGISVYMFNLALADFLY-VLTLPALIFYYFNK  103
RDC1        YIFIFVIGMIANSVVVWVN--IQAKTTGYDTHCYILNLAIADLWV-VVTIPVWVVSLVQH  107
ANG II      YSIIFVVGIFGNSLVVIVI--YFYMKLKTVASVFLLNLALADLCF-LLTLPLWAVYTAME  91
Thrombin    YTGVFVVSLPLNIMAIVVF--ILKMKVKKPAVVYMLHLATADVLF-VSVLPFKISYYFSG  165
PAF         YSIIFVLGIIANGYVLWVFARLYPSKKLNEIKIFMVNLTVADLLF-LITLPLWIVYYSNQ  80
A1          EVLIALVSVPGNVLVIWAV--KVNQALRDATFCFIVSLAVADVAVGALVIPLAILINIGP  73
A2          ELAIAVLAILGNVLVCWAV--WLNSNLQNVTNYFVVSLAAADIAVGVLAIPFAITISTGF  70

P2Y1        TDWIFGDVMCKLQRFIFHVNLYGSILFLTCISVHRYTGVVHPLKSLGRLKKKNAVYVSSL  163
RDC1        NQWPMGELTCKITHLIFSINLFGSIFFLTCMSVDRYLSITYFASTSSRRKKVVRRAVCVL  167
ANG II      YRWPFGNYLCKIASASVSFNLYASVFLLTCLSIDRYLAIVHPMKSRLRRTMLVAKVTCII  151
Thrombin    SDWQFGSELCRFVTAAFYCNMYASILLMTVISIDRFLAVVYPMQSLSWRTLGRASFTCLA  225
PAF         GNWFLPKFLCNLAGCLFFINTYCSVAFLGVITYNRFQAVKYPIKTAQATTRKRGIALSLV  140
A1          RTYFHT---CLMVACPVLILTQSSILALLAIAVDRYLRVKIPLRYKTVVTPRRAAVAIAG  130
A2          CAACHN---CLFFACFVLVLTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAV  127

P2Y1        VWALVVAVIAPILF----YSGTGVRRNK----TIT-CYDTTADEYLRSYFVY-SMCTTVF  213
RDC1        VWLLAFCVSLPDTY----YLKTVTSASN----NETYCRSFYPEHSVKEWLISMELVSVVL  219
ANG II      IWLLAGLASLPAII----HRNVFFIENT----NITVCAFHYESQN-STLPIGLGLTKNIL  202
Thrombin    IWALAIAGVVPLVL----KEQTIQVPGL----NITTCHDVLNETLLEGYYAYYFSAFSAV  277
PAF         IWVAIVAAASYFLV----MDSTNVVSNKAGSGNITRCFEHYEKGSKPVLII---HICIVL  193
A1          VWILSFVVGLTPLFGWNRLGEAQRAWAANGSGGEPVIKCEFEKVISMEYMVYFNFFVWVL  190
A2          CWVLSFAIGLTPMLGWNNCSQPKEGRNYSQGCGEGQVACLFEDVVPMNYMVYYNFFAFVL  187

P2Y1        M-FCIPFIVILGCYGLIVKALIYK------DLDNSPL--RRKSIYLVIIVLTV-FAVSYL  263
RDC1        G-FAIPFCVIAVFYCLLARAISAS-----SDQEKQSS--R-----KIIFSYVVVFLVCWL  266
ANG II      G-FLFPFLIILTSYTLIWKALKKA-----YEIQKNKP--RNDDIFKIIMAIVLFFFFSWI  254
Thrombin    F-FFVPLIISTVCYVSIIRCLS-S-----SAVANRSK--KSRALFLSAAVFCI-FIICFG  327
PAF         G-FFIVFLLILFCNLVIIHTLLRQ-----PVKQQRNAEVRRRALWMVCIVLAV-FVICFV  246
A1          PPLLLMVLIYLEVFYLIRRQLGKKVSAS-SGDPQKY-YGKELKIAKSLALILFLFALSWL  248
A2          VPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVGLFALCWL  247

P2Y1        PFHVMKTLNLRARLDFQTPQMCAFNDKVYATYQVTRGLASLNSCVDPILYFLAGDTFRRR  323
RDC1        PYHVVVLLDIFSILHY-IPFTCQLENFLFTALHVTQCLSLVHCCVNPVLYSFINRNY-RY  324
ANG II      PHQIFTFLDVLIQLGI-IR-DCRIADIVDTAMPITICIAYFNNCLNPLFYGFLGKKFKRY  312
Thrombin    PTNVL----LIAHYSFLSHT--STTEAAYFAYLLCVCVSSISSCIDPLIYYYASSECQRY  381
PAF         PHHMVQLPWTLAELGMWPS---SNHQAINDAHQVTLCLLSTNCVLDPVIYCFLTKKFRKH  303
A1          PLHILNCITLFCP-------SCRKPSI-L--MYIAIFLTHGNSAMNPIVYAFRIQKFRVT  298
A2          PLHIINCFTFFCP-------ECSHAPLWL--MYLTIVLSHTNSVVNPFIYAYRIREFRQT  298

P2Y1        LSRATRKSSRRSEPNVQSKSEEMTLNILTEYKQNGDTSL                       362
RDC1        ELMKAFIFKYSAKTGLTKLIDASRVSE-TEYSALEQNAK                       362
ANG II      FLQ---LLKYIPPKAKSHSNLSTKMSTLSYRPSDNVSSSTKKPAPCFEVE            359
Thrombin    VYSILCCKESSDPSSYNSSGQLMASKMDTCSSNLNNSIYKKLLT                  425
PAF         LSEKLNIMRSSGKCSRVTTDTGTEMAIPINHTPVNPIKN                       342
A1          FLKIWNDHFRCQPTPPVDEDPPEEAPHD                                  326
A2          FRKIIRSHVLRRREPFKAGGTSARALAAHGSDGEQISLRLNGHPPGVWANGSAPH-(58)  411
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Fig. 3. Comparison of the chick P_{2Y1} purinoceptor with the canine orphan RDC1, human angiotensin II type 1 (ANG II), human thrombin, guinea pig platelet activating factor (PAF) and canine adenosine, A1 and A2 receptors. Numbers in parentheses indicate the amino acids removed, and dashes the gaps inserted, to optimise the alignment using the Clustal program [29] (v1.0). Identical results are indicated in bold type.

$CaCl_2$, pH 7.4. Resting potentials varied from −23 to −65 mV and input resistances from 0.5 to 2 MΩ. $I_{Cl(Ca)}$ was activated under voltage clamp, using a step-pulse protocol to establish whether antagonists interfered with the endogenous calcium-activated chloride channel [13] in the oocyte. A command step to −100 mV for 1 s was followed by a depolarising command for 4 s to +50 mV to activate $I_{Cl(Ca)}$, before returning to the holding potential of −40 mV. This protocol was repeated every 20 s in the absence and presence of the antagonists. The measured current relaxation was indeed $I_{Cl(Ca)}$ since when external Ca was removed and equimolar $BaCl_2$ was applied the current was substantially reduced.

2.3. *Hybridization analysis of chicken RNA*

Total cellular RNA from a variety of one-day-post-hatch chick and chicken tissues was prepared by CsCl centrifugation [12]. Poly(A)$^+$-selected RNA was prepared by oligo(dT) cellulose chromatography. RNA was electrophoresed through a 1% formaldehyde 0.8% agarose gel. Following electrophoresis, RNA was blotted to Hybond-N nylon membrane (Amersham) and hybridisation was performed at 42°C in 50% formamide [12]. The hybridisation probe was an antisense 45-base oligonucleotide corresponding to the DNA sequence encoding amino acids 1–15 of 803 and was labelled by terminal deoxynucleotidyl transferase to a specific activity of approximately 1×10^9 dpm/μg. The filter was washed at 65°C in 1 × SSC, 0.1% SDS for 15 min before being exposed to an X-ray film at −70°C with an intensifying screen. Exposure: 10 days (1 day for chick brain).

3. RESULTS AND DISCUSSION

A guinea pig partial cDNA [9] was used to screen 5×10^5 recombinants of an embryonic chick whole brain cDNA library. Sequence analysis of one of the isolated clones, 803, revealed the presence of an open reading frame of 1,068 bp. The predicted amino acid sequence and the nucleotide sequence are presented in Fig. 1. The putative initiator methionine is in agreement with the optimal sequence for translation [14] and the presence of an in-frame stop codon upstream of this methionine codon indicates that 803 encodes the entire coding region of a polypeptide of 362 amino acids with a calculated molecular weight of 41 kDa. The hydrophobicity profile [15] (not shown) of the predicted amino acid sequence revealed the typical pattern of a GCR and exhibited seven hydrophobic domains. The sequence possesses other common features [16] of the GCR superfamily, including: (a) consensus sequences for N-linked glycosylation near the amino terminus and in the first two extracellular loops; (b) two conserved cysteine residues, one in each of the first two extracellular loops, that are believed to form a disulphide bond which stabilises the functional protein structure; (c) serine and threonine residues in the third cytoplasmic loop and COOH-terminal domain, which represent potential phosphorylation sites and may play a role in receptor desensitization (Fig. 1).

To establish the identity of the 803 protein, *Xenopus laevis* oocytes were injected with in vitro transcribed cRNA prepared from clone 803 [7,8]. ATP (1 μM–1 mM) caused a slowly-developing inward current associated with a conductance increase, in cRNA-injected oocytes (Fig. 2Aa). The slow but large, concentration-dependent outward currents and conductance increases, which can be seen in non-injected control oocytes, representing activation of native adenosine receptors, were completely inhibited by bath application of 100 μM theophylline [17]. Therefore, all experiments were performed in the presence of theophylline, which does not interfere with responses to P_2 receptor activation [17]. After inhibition with theophylline, reapplication of 100 μM ATP did not reveal any previously occluded oscillatory inward current in control oocytes. Membrane current and conductance oscillated during and for some time after agonist application. The observed responses, together with a reversal potential of −24 mV, indicated the involvement of a Ca-activated Cl^- current ($I_{Cl(Ca)}$) [13] which is normally evoked in the oocyte by expressed GCRs [18]. Responses to ATP were dose-dependent, with a threshold concentration of approximately 10 μM and an EC_{50} (half-maximal concentration) of 49.5 ± 6 μM (Fig. 2Aa,2B). Repeated applications of the same concentration of ATP produced consistent responses, with a negligible decline in response amplitude over time. Inward currents were also induced by 2-methylthio-ATP (2-MeSATP) and ADP. In contrast, UTP and the P_{2X}-selective agonists [2] α,β-methylene-ATP (α,β-MeATP), and β,γ-methylene-ATP (β,γ-MeATP) were inactive at concentrations up to 30–100 μM (Fig. 2A). The relative order of agonist potency (as judged by matched-response amplitudes) was: 2-MeSATP ≥ ATP > ADP ≫ α,β-MeATP, β,γ-MeATP, UTP. This specificity was found likewise in radioligand binding studies performed on chick brain membranes, or on 803 transfected cos-7 cells, with a high-affinity binding site for ATP being present (to be reported elsewhere). In injected oocytes both suramin (100 μM) and the P_{2Y}-selective antagonist [19] Reactive Blue 2 (RB) (10 μM) antagonised the responses to ATP and to 2-MeSATP (Fig. 2Ca,Cb) without appreciably affecting native $I_{Cl(Ca)}$. The high potency of ATP and the inactivity of the methylene ATPs and of UTP identified this receptor as a P_{2Y} subtype rather than a P_{2X} or P_{2U} subtype. The near equipotency of 2-MeSATP and ATP, and greater potency of ATP over ADP, suggested that this expressed receptor is a novel subtype of the P_{2Y} purinoceptor family. We have therefore designated this receptor as P_{2Y1}.

Fig. 4. Tissue distribution of the transcript corresponding to the P_{2Y1} receptor. A Northern blot of chick (one-day-post-hatch) whole brain poly(A)$^+$ (10 μg) and total RNA (30 μg) from various chick and adult tissues was subjected to Northern analysis as described in Section 2. Tissues were: Bc, chick brain; Hc, chick heart; Lc, chick liver; and from adult chicken. B, brain; H, heart; L, liver; G, gastrointestinal tract; ST, stomach; SP, spleen; K, kidney; SC, spinal cord; LU, lung; LM, leg muscle. Band size (arrow) was determined using RNA standards (BRL). Bands were absent from the regions of the blot not shown

Comparison of the P_{2Y1} purinoceptor with other cloned GCR sequences places it in the major family of these receptors [20]: negligible sequence identity was seen with either the secretin/calcitonin or the glutamate receptor gene families. P_{2Y1} shares no more than 27% identity with known GCR sequences (Table I), suggesting that this receptor protein is in a previously unknown subdivision of this major receptor family. It is of interest that the P_{2Y1} receptor has only a low sequence identity with the adenosine [10] (21%) and cAMP [21] (17%) receptors (Table I, Fig. 3). The P_{2Y1} receptor sequence does not contain ATP binding motifs [22] of the type $G[X]_4GK$. The absence of a linear ATP binding motif is not surprising, since it is likely that the ligand-binding pocket for a molecule the size of ATP will be formed by residues in several transmembrane domains as in the case of the catecholamine receptors [23].

The tissue distribution of the P_{2Y1} transcript was determined by Northern hybridisation. The mRNA (3.1-kb) had a discrete pattern of expression in the adult chicken: it was present in brain, spinal cord, gastrointestinal tract, spleen and leg muscle (Fig. 4) but was not detected in heart, liver, stomach, lung or kidney. The transcript was also found in the brain of newly-hatched chick (Fig. 4). P_{2Y} purinoceptors are abundant in the mammalian gastrointestinal tract and spinal cord [2] but their distribution in chicken tissues is unknown. However, in developing chick skeletal muscle P_2 purinoceptors have been found in physiological studies, but not of the defined P_{2Y} type [24]. The existence of P_{2Y} purinoceptors in mammalian liver, kidney, pancreas, blood vessels and heart has also been reported [2,25–27], but the observed pharmacology of these receptors differ [5] from the purinoceptor described here. Our data support the hypothesis that the chick P_{2Y1} purinoceptor is distinct from the P_{2Y} purinoceptors described previously in visceral tissues.

ATP is known to act as a fast neurotransmitter in peripheral and central neurones, via P_{2X} purinoceptor cation channels [5,28]. The presence of P_{2Y1} purinoceptors in the brain indicates the involvement of ATP in metabotropic, slow synaptic transmission. Indeed, the high concentration of the P_{2Y1} mRNA and the exceedingly high and specific binding capacity for ATP in the developing chick brain (J.S. and T.E.W., unpublished) is indicative of a more significant role for a P_{2Y} purinoceptor in the brain than has hitherto been suspected.

Table I

Percentage identity of chick P_{2Y1} purinoceptor with known G-protein-coupled receptors

Receptor	Species	% Identity with P_{2Y1}
RDC1	canine	27
Angiotensin II type 1	human	27
Thrombin	human	25
Platelet activating factor	guinea pig	25
C5a anaphylatoxin	human	23
Neuromedin K	rat	23
Interleukin 8	human	22
Bradykinin B2	rat	22
Neurotensin	rat	21
Endothelin B	human	21
Gastrin-releasing peptide	mouse	21
Adenosine A1	canine	21
Substance P	human	20
Neurokinin 2	human	20
Adenosine A2	canine	18
cAMP	slime mold	17

The most related sequences are shown, along with adenosine and cAMP receptors. Amino acid sequences were obtained from the Protein Identification Resource (PIR) and were aligned using the Clustal program [29] (v1.0).

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REFERENCES

[1] Burnstock, G. (1972) Pharmacol. Rev. 24, 509–581.
[2] Burnstock, G. (1991) Nucleosides and Nucleotides 10, 917–930.
[3] Krishtal, O.A., Marchenko, S.M., Obukhov, A.G. and Volkova, T.M. (1988) Br. J. Pharmacol. 95, 1057–1062
[4] O'Connor, S.E., Dainty, I.A and Leff, P (1991) Trends Pharmacol. Sci. 12, 137–141
[5] Benham, C.D. and Tsien, R.W. (1987) Nature 328, 275–278.
[6] Jeffs, R.A., Cooper, C.L. and Harden, T.K. (1991) Mol. Pharmacol. 40, 85–92.
[7] Barnard, E.A., Beeson, D., Bilbe, G., Brown, D.A., Constanti, A., Houamed, K. and Smart, T.G. (1984) J. Recep. Res. 4, 681–704.

[8] Smart, T.G., Constanti, A., Bilbe, G., Brown, D.A. and Barnard, E.A. (1983) Neurosci. Lett. 40, 55–59.
[9] Webb, T.E., Bateson, A.N., Barnard, E.A., paper presented at the 17th EMBO Annual Symposium, Heidelberg, Germany, 16 September 1991, Abstract number 208.
[10] Libert, F., Parmentier, M , Lefort, A., Dinsart, C., Van Sande, J., Maenhaut, C., Simons, M., Dumont, J.E. and Vassart, G. (1989) Science 244, 569–572.
[11] Bateson, A.N., Harvey, R.J., Wisden, W., Glencorse, T.A., Hicks, A.A., Hunt, S.P., Barnard, E.A. and Darlison, M.G. (1991) Mol. Brain Res. 9, 333–339.
[12] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, Cold Spring Harbor Laboratory, New York.
[13] Barish, M.E. (1982) J. Physiol. 342, 309–325.
[14] Kozak, M. (1986) Cell 44, 283–292.
[15] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[16] Probst, W.C., Snyder, L.A , Schuster, D.L., Brosius, J and Sealfon, S.C. (1992) DNA and Cell Biology 11, 1–20.
[17] Lotan, I., Dascal, N., Cohen, S. and Lass, Y. (1986) Pflügers Arch. 406, 158–162.
[18] Smart, T.G. (1989) Cell and Mol. Neurobiol. 9, 193–206.
[19] Burnstock, G. and Warland, J.J.I. (1987) Br. J. Pharmacol. 90, 383–391.
[20] Barnard, E.A. (1992) Trends Biochem. Sci. 17, 368–374.
[21] Klein, P., Sun, T.J., Saxe, C.L., Kimmel, A.R. and Devreotes, P.N (1988) Science 241, 1467–1472.
[22] Saraste, M., Sibbald, P.R. and Wittinghofer, A. (1990) Trends Biochem. Sci 15, 430–4300.
[23] Dixon, R.A.F., Sigal, I.S., Candelore, M.R., Register, R.B., Scatterwood, W., Rands, E. and Strader, C.D. (1987) EMBO J. 6, 3269–3275.
[24] Thomas, S.A., Zawisa, M.J., Lin, X. and Hume, R.I. (1991) Br. J. Pharmacol. 103, 1963–1969.
[25] Dixon, C.J., Woods, N.M., Cuthbertson, K.S.R. and Cobbold, P.H. (1990) Biochem. J. 269, 499–502.
[26] Zhau, D. and Dhalla, N.S. (1990) Am. J. Physiol. 258, C185–189.
[27] Pfeilschifter, J. (1990) Biochem. J. 272, 469–472.
[28] Edwards, F.A., Gibb, A.J. and Colquhoun, D. (1992) Nature 359, 144–147.
[29] Higgins, D.G. and Sharp, P.M. (1989) CABIOS 5, 151–153.