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Functional α_{2C}-adrenoceptors in human neuroblastoma SH-SY5Y cells

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Abstract

The α_2-adrenoceptor mediating inhibition of forskolin-stimulated cyclic AMP accumulation in human neuroblastoma SH-SY5Y cells was further characterized. The α_2-adrenoceptor agonists, UK 14,304 (5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline), oxymetazoline, guanfacine, (−)-noradrenaline and clonidine concentration-dependently decreased cyclic AMP accumulation in this cell line (E_{max} ca. 50% inhibition). Agonist pEC_{50} values ranged between 6.7 and 7.8. Clonidine was a partial agonist. The effects of UK 14,304 were blocked after a pertussis toxin treatment. The concentration–response curves of UK 14,304 were shifted to the right in a parallel manner by the following antagonists (mean pK_B values): yohimbine (8.17), idazoxan (7.63), prazosin (6.66), 2-[2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl]-4,4-dimethyl-1,3-(2H,4H) isoquinolindione (ARC 239; 7.12) and 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB-4101; 8.12). The relatively high pK_B values of prazosin and ARC 239 point to a non-α_{2A}-adrenoceptor-mediated effect. The relatively high pK_B value of WB-4101 further characterizes the α_2-adrenoceptor in SH-SY5Y cells as being of the α_{2C} subtype. The analysis of the expression of α_2-adrenoceptor subtypes by reverse transcriptase-polymerase chain reaction (RT-PCR) revealed the exclusive presence of α_{2C}-adrenoceptor mRNA in SH-SY5Y cells. We propose that inhibition of forskolin-stimulated cAMP accumulation in SH-SY5Y cells be used as a functional model of human, native α_{2C}-adrenoceptors.

Keywords: α_2-Adrenoceptor; α_{2C}-Adrenoceptor; SH-SY5Y cell, human; Neuroblastoma cell, human; cAMP

1. Introduction

α_2-Adrenoceptors constitute a distinct family of catecholamine receptors which basically consists of three homologous subtypes (α_{2A}, α_{2B} and α_{2C}; Bylund, 1988). The genes encoding all three human subtypes have been cloned and expressed in heterologous systems (Kobilka et al., 1987; Regan et al., 1988; Lomasney et al., 1990). The α_{2A}-adrenoceptor subtype undergoes species variations resulting in a distinct pharmacology in rodents, previously referred to as α_{2D} (see Hieble et al., 1995).

α_2-Adrenoceptors belong to the G-protein coupled receptor superfamily. In recombinant systems, their preferential transduction pathway is through inhibitory, pertussis toxin sensitive G_i/G_o proteins, although G_s-mediated effects have been reported as well, particularly in the case of α_{2B}-adrenoceptors (Eason et al., 1992; Pohjanoksa et al., 1997). Well characterized models of native α_2-adrenoceptor subtypes, functionally linked to inhibition of cyclic AMP (cAMP) accumulation have been found in various cell lines. As far as human receptors are concerned, HT29 human colonic adenocarcinoma cells are one of the few cell lines containing a pure population of an α_2-adrenoceptor subtype (α_{2A}; Bylund and Ray-Prenger, 1989). NG108-15 neuroblastoma × glioma rat-mouse hybrid cells (Bylund and Ray-Prenger, 1989) and OK American opossum kidney cells (Murphy and Bylund, 1988) are regarded as sources of α_{2B}- and α_{2C}-adrenoceptor subtypes, respectively. However, they do not have human counterparts which could be used for functional and pharmacological studies.

In the following, we report on the pharmacological characterization of α_2-adrenoceptors mediating inhibition of cAMP accumulation in human neuroblastoma SH-SY5Y cells. Evidence is presented for them to be of the α_{2C}-adrenoceptor subtype. This was supported by mRNA analysis by reverse transcriptase-polymerase chain reaction (RT-PCR), showing the presence of α_{2C}-adrenoceptor mRNA in SH-SY5Y cells, whereas transcripts encoding

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the α_{2A}- and the α_{2B}-adrenoceptor subtypes were not detected.

2. Materials and methods

2.1. Cell culture

Human neuroblastoma SH-SY5Y cells were grown in Dulbecco's modified Eagle medium supplemented with 10% foetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. They were split twice a week using a trypsin/EDTA solution.

2.2. cAMP measurements

Cells grown to confluence in 24-well plates were deprived of serum 24 h before the experiments. cAMP accumulation was measured in intact cells using the standard [^{3}H]adenine prelabelling technique, as previously described (Schoeffter et al., 1997). The [^{3}H]cAMP/([^{3}H]cAMP + [^{3}H]ATP) cpm ratio was calculated for each sample. Inhibition of forskolin-stimulated cAMP accumulation was normalised to the effect of forskolin (10 μM). Concentration–response curves were fitted to the logistic function $f(x) = a/(1 + b/x)$ using the RS/1 program package (Bolt, Beranek and Newton, Boston, MA, USA), wherefrom a (E_{max}) and b (EC_{50}) values were derived. In case an antagonist showed intrinsic activity, the latter was taken into account in the calculations, i.e., subtracted from the total effect. Results are given as means ± S.E.M. from n experiments, each made in duplicate. Apparent pK_B values of antagonists were calculated from the rightward shift of the agonist concentration–response curve, according to the formula: $pK_B = \log$ (ratio-1) − log molar [antagonist], wherein ratio designates the ratio (EC_{50} in the presence/EC_{50} in the absence of antagonist).

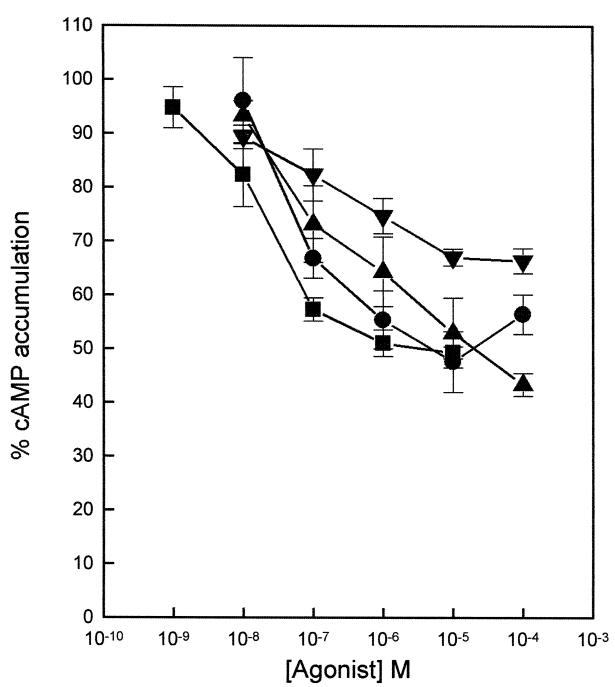

Fig. 1. Concentration–response curves of UK 14,304 (■), oxymetazoline (●), guanfacine (▲) and clonidine (▼) for inhibition of forskolin-stimulated cAMP accumulation in SH-SY5Y cells. Points represent mean values ± S.E.M. (vertical bars) of five, four, five and three experiments, respectively.

Table 1
Agonist parameters in SH-SY5Y cells

Agonist	% Maximal inhibition	pEC_{50}	n
UK 14,304	50 ± 2	7.83 ± 0.17	5
Oxymetazoline	47 ± 3	7.37 ± 0.20	4
Guanfacine	53 ± 1	6.71 ± 0.50	5
(−)-Noradrenaline	45 ± 3	7.29 ± 0.37	4
Clonidine	32 ± 1	7.20 ± 0.40	3

2.3. mRNA analysis

Total RNA was isolated from the SH-SY5Y cell line by using the S.N.A.P.™ Total RNA Isolation Kit (Invitrogen, Catalog No. K1950-01). To eliminate any contaminating genomic DNA, the RNA sample was treated twice with RNAse-free DNAseI. Two micrograms of total RNA were transcribed into the first strand of cDNA in 30 μl of buffer containing 1 μM oligo dT primer, 10 mM dithiothreitol, 0.6 mM 2′-deoxyribonucleotides, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$. The reverse transcriptase reaction mix without the reverse transcriptase was incubated at 65°C for 10 min, and then at 37°C for 5 min. Two hundred units of reverse transcriptase (Gibco/BRL) were added and the reaction was incubated at 37°C for 60 min. The reaction was stopped by heating up to 95°C for 10 min. For PCR, primer pairs were designed for specific amplification of the α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor subtype, respectively. In each case, the identity of the PCR product was determined by molecular size and sequence analysis. The α_{2A} amplification (forward primer: 5′-GGC AAG ACT TGG TGC GA-3′; reverse primer: 5′-CGA CGA GAT GAC GTA CCA CT-3′) generates a PCR fragment of 297 bp, whereas the α_{2B} (forward primer: 5′-CCT GCG CAT CTA CCT GAT CG-3′; reverse primer: 5′-AAG CTG AGG CCG GAG ACA CT-3′) and α_{2C} (forward primer: 5′-GTG GTG ATC GCC GTG CTG AC-3′; reverse primer: 5′: CGT TTT CGG TAG TCG GGG AC-3′) yield PCR fragments of 391 bp and 574 bp in length, respectively. For each PCR amplification, 1/10 of the reverse transcriptase reaction (3 μl) was used as template. The analysis of the expression of α_{2A}, α_{2B} and α_{2C} mRNA was carried out as follows: briefly, the PCR amplification was performed in a buffer of 20 μl containing 3 μl of the reverse transcriptase reaction, 130 μM 2′-deoxy-

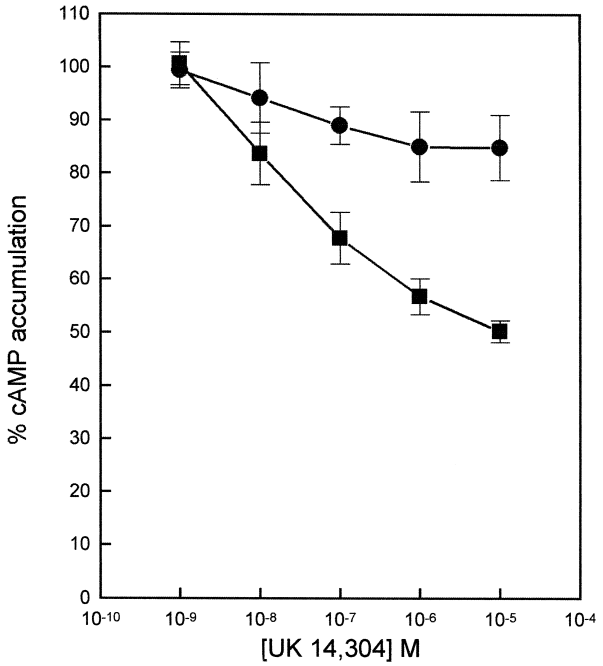

Fig. 2. Concentration–response curves of UK 14,304 for inhibition of forskolin-stimulated cAMP accumulation in pertussis toxin-treated (●) and control (■) SH-SY5Y cells. Points represent mean values ± S.E.M. (vertical bars) of six experiments.

ribonucleotides, 1 × PCR buffer (Pharmacia), 0.5 μM each of the two primers and 1 U Taq polymerase (Pharmacia). The PCR amplification took place in a Biometra thermocycler with the following cycles: 1 ×: 2 min at 95°C; 30 ×: 45 s at 95°C, 45 s at 65°C, 60 s at 72°C; 1 ×: 7 min at 72°C. The PCR products were size fractionated on a 1.5% agarose gel and visualized by ethidium bromide staining.

2.4. Drugs

The substances were obtained from the following sources: UK 14,304 (5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline), WB-4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) and ARC 239 (2-[2-(4-(2-methoxyphenyl)piperazin-1-yl) ethyl]-4,4-dimethyl-1,3-(2H,4H) isoquinolindione dihydrochloride) from Tocris (Bristol, England); clonidine hydrochloride, yohimbine hydrochloride and pertussis toxin from Sigma (Fluka, Buchs, Switzerland); oxymetazoline hydrochloride from Merck (Darmstadt, Germany); idazoxan from Reckitt and Colman (Kingston upon Hull, England); (−)-noradrenaline bitartrate from Research Biochemicals International (Natick, MA, USA). Guanfacine hydrochloride and prazosin hydrochloride came from internal sources at Novartis Pharma (Basel, Switzerland). The substances were prepared daily at 10^{-2} M concentrations in distilled water, except for UK 14,304 which was dissolved in DMSO and prazosin which was dissolved in a mixture of 1-methyl-2-pyrrolidine:ethanol (1:1) containing 20 mg/ml ascorbic acid, and further diluted with water.

3. Results

3.1. Agonist studies

α_2-Adrenoceptor agonists concentration-dependently decreased the cAMP accumulation induced by forskolin

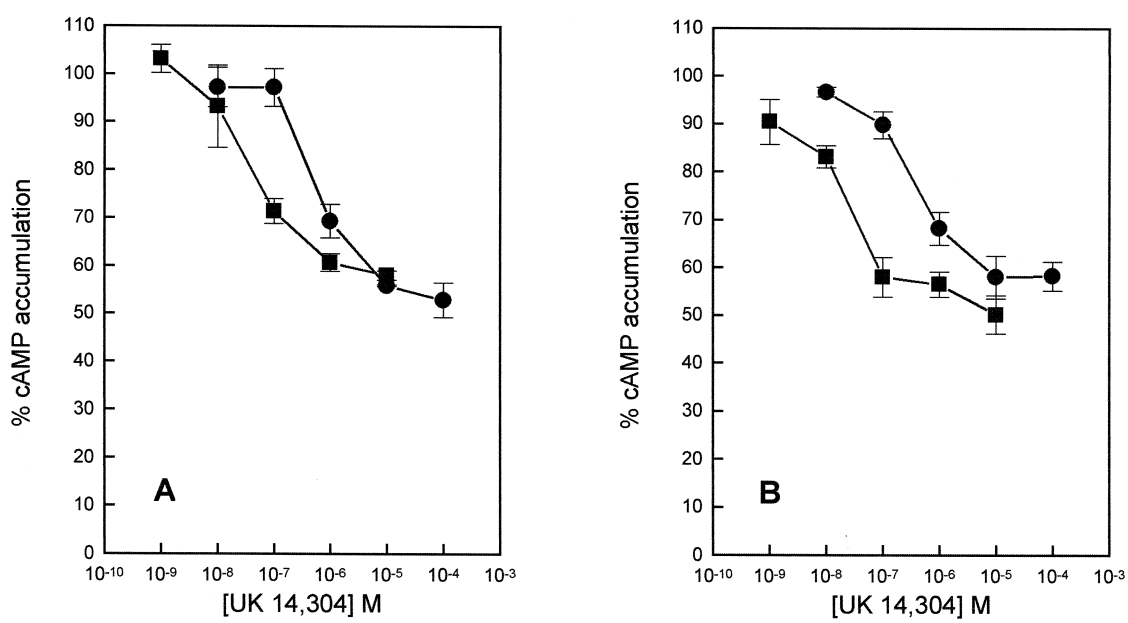

Fig. 3. Concentration–response curves of UK 14,304 for inhibition of forskolin-stimulated cAMP accumulation in SH-SY5Y cells, in the absence (■) and in the presence (●) of (A) yohimbine (0.1 μM) and (B) idazoxan (1 μM). Points represent mean values ± S.E.M. (vertical bars) of three or four experiments.

Table 2
Antagonist parameters in SH-SY5Y cells

Antagonist	pK_B	*n*
Yohimbine	8.17 ± 0.12	3
Idazoxan	7.63 ± 0.44	4
Prazosin	6.66 ± 0.12	8
ARC 239	7.12 ± 0.15	6
WB-4101	8.12 ± 0.18	18

(10 μM) in SH-SY5Y cells (Fig. 1). Maximal inhibition was about 50%. The most potent agonist was UK 14,304 (mean pEC_{50} value 7.83). Oxymetazoline and guanfacine mimicked the effects of UK 14,304 with the same E_{max} but somewhat less potency (Fig. 1; Table 1). (−)-Noradrenaline was also a full agonist compared to UK 14,304 (Table 1). Clonidine clearly behaved as a partial agonist (E_{max} 32% inhibition). The effects of UK 14,304 were blocked by a pre-treatment of the cells with pertussis toxin (100 ng/ml for 18–24 h) (Fig. 2).

3.2. Antagonist studies

3.2.1. Non-subtype selective α_2-adrenoceptor antagonists

The concentration–response curve of UK 14,304 was shifted to the right in a parallel manner by the non-subtype selective α_2-adrenoceptor antagonists, yohimbine (0.1 μM) and idazoxan (1 μM), yielding mean pK_B values of 8.17 and 7.63, respectively (Fig. 3; Table 2).

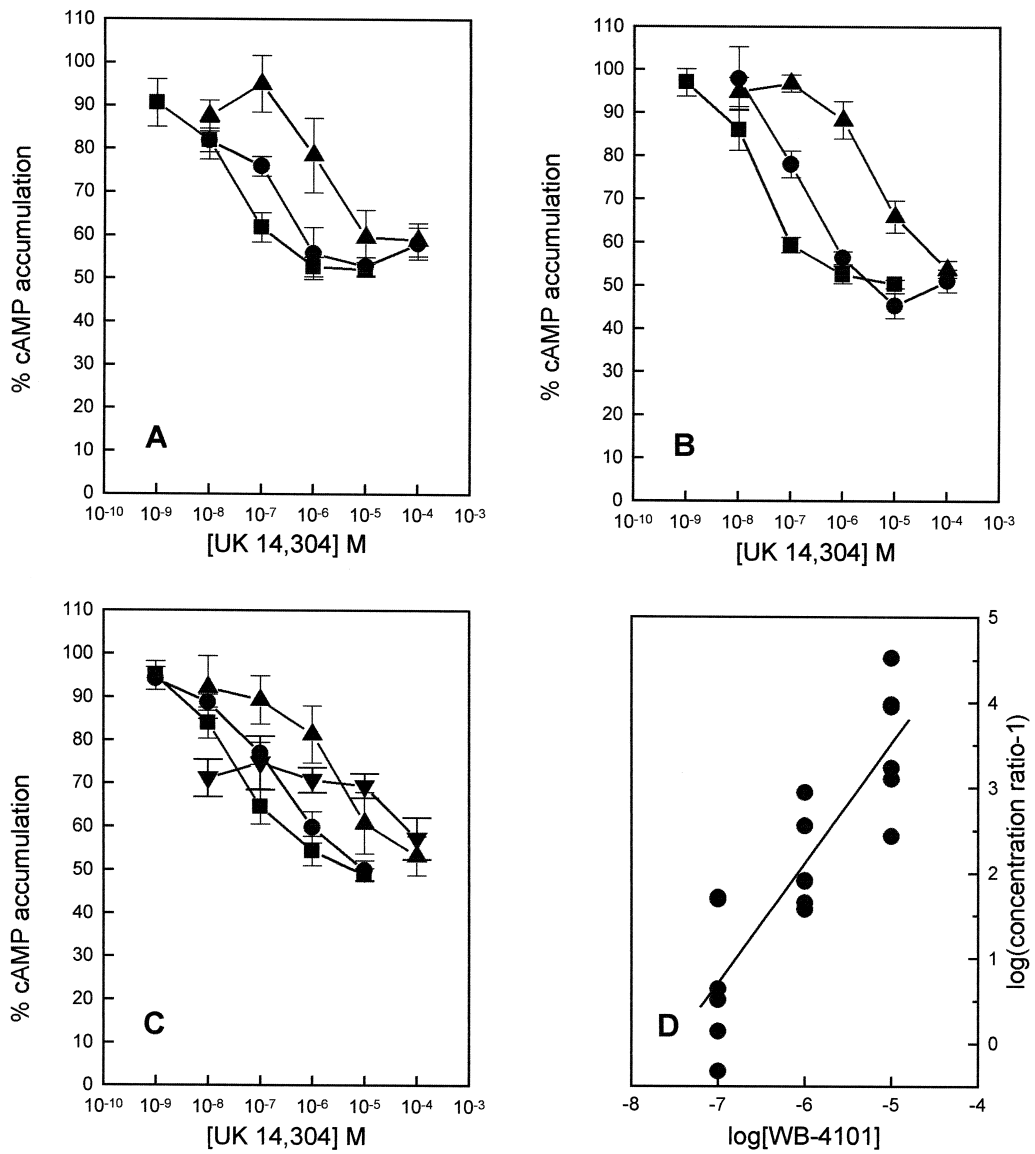

Fig. 4. Concentration–response curves of UK 14,304 for inhibition of forskolin-stimulated cAMP accumulation in SH-SY5Y cells, in the absence (■) and in the presence of (A) prazosin (●: 1 μM and ▲: 10 μM); (B) ARC 239 (●: 1 μM and ▲: 10 μM) and (C) WB-4101 (●: 0.1 μM, ▲: 1 μM and ▼: 10 μM). Points represent mean values ± S.E.M. (vertical bars) of four, three and six experiments with each concentration of prazosin, ARC 239 and WB-4101, respectively. Control curves were pooled. (D) Schild plot of the antagonism of WB-4101 versus UK 14,304.

3.2.2. Subtype selective α_2-adrenoceptor antagonists

Prazosin and ARC 239, two antagonist compounds discriminating between α_{2A}-adrenoceptors on one side and $\alpha_{2B/C}$-adrenoceptors on the other side, were investigated. Prazosin and ARC 239 (1 and 10 μM) produced concentration-dependent, rightward shifts of the UK 14,304 control curve (Fig. 4A and B). The mean pK_B values of prazosin and ARC 239 were 6.66 and 7.12, respectively (Table 2). Compound WB-4101 was tested against UK 14,304 because it has reportedly high affinity for $\alpha_{2A/C}$-adrenoceptors and selectivity over α_{2B}-adrenoceptors. WB-4101 (0.1, 1 and 10 μM) induced concentration-dependent shifts of the UK 14,304 curve to the right, without any depression of the E_{max} (Fig. 4C). However, Schild plot analysis of the antagonism yielded a slope factor significantly departing from unity (1.40 ± 0.20, $n = 18$), hinting towards a non-simple, non-competitive interaction (Fig. 4D). It should also be mentioned that WB-4101 displayed intrinsic activity at the highest concentration tested (10 μM). Averaging the pK_B values obtained with all concentrations of WB-4101 used yielded a mean value of 8.12.

3.3. α_2-Adrenoceptor mRNA analysis

The expression of α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor genes in SH-SY5Y cells was analyzed by using the RT-PCR approach. The primer pairs designed for specific amplification of the three intronless α_2-adrenoceptor subtypes were initially tested for specific PCR amplification by using genomic DNA as template. The PCR amplifications yielded in each case a major PCR product of the expected size (Fig. 5: lanes B1, B2 and B3) and, as shown by sequence analysis, of the correct sequence. To check for contaminating genomic DNA present in the RNA samples, PCR experiments were performed for all three subtypes by using RNA as a template. Any product resulting from these reactions would represent amplified genomic DNA. As shown in Fig. 5 (lanes C1, C2 and C3), none of the α_2 PCR amplifications yielded a DNA product when RNA (without reverse transcription) was used as a template.

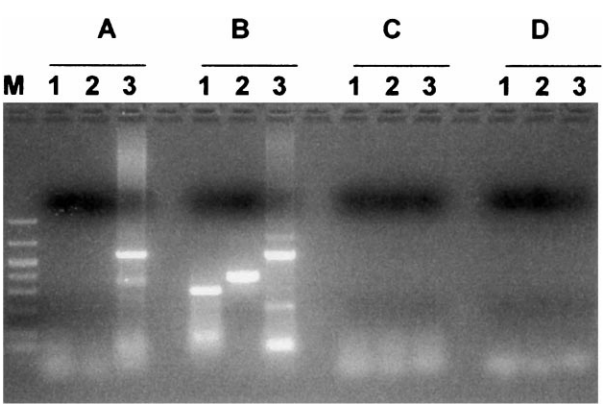

Fig. 5. Expression of α_2-adrenoceptor subtype mRNA in the cell line SH-SY5Y. The amplification products derived from the α_{2A} (lane 1), α_{2B} (lane 2) and α_{2C} (lane 3) polymerase chain reaction (PCR) were fractionated on an agarose gel and visualized by ethidium bromide staining. The templates used for the PCR amplifications are indicated by A (total RNA reverse transcribed into the first strand of cDNA), B (human genomic DNA; positive control), C (total RNA; control for genomic DNA contamination) and D (no template added; negative control). The euroladder® M DNA standard (Eurobio) was used as a DNA molecular weight marker.

The RT-PCR analysis of the expression of the α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor genes clearly showed the exclusive expression of the α_{2C} gene in SH-SY5Y cells (Fig. 5: lanes A1, A2, A3). The identity of the amplified fragment was confirmed by sequence analysis to be of the α_{2C} subtype (GeneBank accession number: J03853). No PCR products were obtained for the α_{2A} and α_{2B} subtypes when reverse transcribed mRNA was used as a template, indicating that the α_{2A} and α_{2B} subtypes are not expressed in SH-SY5Y.

4. Discussion

Kazmi and Mishra (1989) first demonstrated the presence of specific α_2-adrenergic binding sites in SH-SY5Y cells. Using [^{3}H]rauwolscine as a radioligand, they found a B_{max} of 44 fmol/mg protein. Lameh et al. (1992) found these sites to be functionally coupled to inhibition of cAMP accumulation. However, these functional α_2-adrenoceptors present in SH-SY5Y have not been typified as yet, in terms of cloned human α_{2A}-, α_{2B}- or α_{2C}-adrenoceptors. The present work shows evidence that α_2-adrenoceptor-mediated inhibition of cAMP in SH-SY5Y cells is through stimulation of the α_{2C}-adrenoceptor subtype.

As expected from the general profile of all α_2-adrenoceptor subtypes, we found that UK 14,304, oxymetazoline, guanfacine and clonidine (partial agonist), as well as the natural agonist, (−)-noradrenaline produced concentration-dependent decreases of forskolin-stimulated cAMP accumulation. These effects were G_i protein-mediated, as shown by their sensitivity to pertussis toxin (pertussis toxin causes receptor/G_i protein uncoupling by ADP-ribosylating this type of G protein at the level of a Cys residue located four aminoacids from the carboxyl terminus; West et al., 1985).

None of the agonists, however, is sufficiently selective to conclude with regard to the subtype involved. Even though UK 14,304 and oxymetazoline have been proposed to show some selectivity (MacDonald et al., 1997), this is of no real help since agonists may have spare receptors which can strongly influence their potencies in a given functional assay. Along the same lines of reasoning, the antagonism by yohimbine and idazoxan, while confirming the presence of functional α_2-adrenoceptors in SH-SY5Y cells, did not permit further subclassification. Yohimbine and idazoxan are prototype α_2-adrenoceptor antagonists without marked subtype selectivity.

To further characterize the α_2-adrenoceptor subtype in SH-SY5Y cells, we took advantage of the fact that prazosin and ARC 239 are able to discriminate between the α_{2A}-adrenoceptor versus the $\alpha_{2B/C}$-adrenoceptors. Thus, radioligand binding pK_i values well below 6 are reported for these two compounds at human recombinant α_{2A}-adrenoceptors, whereas corresponding values at α_{2B}- and α_{2C}-adrenoceptors are close to 7 (Regan et al., 1988; Lomasney et al., 1990; Marjamäki et al., 1993; Devedjian et al., 1994; Uhlen et al., 1994). The functional pK_B values we obtained for prazosin and ARC 239 were 6.66 and 7.12, respectively. This concludes that the SH-SY5Y receptor is not the α_{2A}-adrenoceptor subtype.

However, the known affinities of prazosin and ARC 239 for the α_{2B}- and α_{2C}-adrenoceptors are not sufficiently different to be able to distinguish between the two. Therefore, there was a need to employ a third selective antagonist to discriminate between the α_{2B}- and α_{2C}-adrenoceptors. The antagonist of choice was WB-4101 because it has more than a log difference in its affinities in favour of the human recombinant α_{2C}- over the α_{2B}-adrenoceptor (Lomasney et al., 1990; Devedjian et al., 1994; Uhlen et al., 1994). Our results show a mean pK_B value of 8.12 ± 0.18 for WB-4101 in SH-SY5Y, which lies in the range of values reported at human recombinant α_{2C}-adrenoceptors (7.89–8.50) but not in that of α_{2B}-adrenoceptors (6.88–7.11). We therefore conclude that the cAMP inhibitory responses to α_2-adrenoceptor agonists in SH-SY5Y cells are mediated mostly if not entirely by α_{2C}-adrenoceptors. The non-compliance of the Schild regression with the Schild equation describing a straight line of slope equal to unity may indicate that antagonism by WB-4101 is not a simple, competitive one. Alternatively, involvement of another (minor) α_2-adrenoceptor subtype may be invoked. One should also keep in mind that WB-4101 showed intrinsic activity at the highest concentration tested (10 μM); this could have biased the Schild analysis.

The exclusive presence of mRNA encoding the α_{2C}-adrenoceptor subtype in SH-SY5Y cells corroborates the pharmacological analysis. As a whole, the results strongly suggest that the responses to α_2-adrenoceptor agonists in these cells are mediated by α_{2C}-adrenoceptors.

Whereas human cell lines expressing native, functionally characterized α_{2A}-adrenoceptors have been identified (e.g., the human colon carcinoma cell line HT29; Bylund and Ray-Prenger, 1989), this is not the case of α_{2B}- and α_{2C}-adrenoceptors. Sources of native α_{2B}- and α_{2C}-adrenoceptors are usually NG108-15 cells (murine neuroblastoma cross rat glioma hybrid cells) and OK cells (American opossum kidney epithelial cells), respectively. Radioligand binding studies have detected the presence of α_{2C}-adrenoceptors in the human retinoblastoma Y79 cell line (Gleason and Hieble, 1992), the hepatoma HepG2 and the neuroblastoma SK-N-MC cell lines (Schaak et al., 1997). In the latter two cases, the presence of α_{2C}-adrenoceptor mRNA has been demonstrated. It is not known, however, whether these lines are suitable for a functional assay. SH-SY5Y cells seem to be unique in this respect.

In conclusion, we propose that inhibition of forskolin-stimulated cAMP accumulation in SH-SY5Y cells be used as a functional model of human, native α_{2C}-adrenoceptors. This may offer an alternative to more heterogeneous tissue models, like human saphenous vein (Gavin et al., 1997) or human kidney slices (Trendelenburg et al., 1994).

References

Bylund, D.B., 1988. Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol. Sci. 9, 356–361.

Bylund, D.B., Ray-Prenger, C., 1989. Alpha-2A and alpha-2B adrenergic receptor subtypes: attenuation of cyclic AMP production in cell lines containing only one receptor subtype. J. Pharmacol. Exp. Ther. 251, 640–644.

Devedjian, J.C., Esclapez, F., Denis-Pouxviel, C., Paris, H., 1994. Further characterization of human α_2-adrenoceptor subtypes: [^{3}H]RX821002 binding and definition of additional selective drugs. Eur. J. Pharmacol. 252, 43–49.

Eason, M.G., Kurose, H., Holt, B.D., Raymond, J.R., Liggett, S.B., 1992. Simultaneous coupling of α_2-adrenergic receptors to two G-proteins with opposing effects. J. Biol. Chem. 267, 15795–15801.

Gavin, K.T., Colgan, M.P., Moore, D., Shanik, G., Docherty, J.R., 1997. α_2-Adrenoceptors mediate contractile responses to noradrenaline in the human saphenous vein. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 406–411.

Gleason, M.M., Hieble, J.P., 1992. The α_2-adrenoceptors of the human retinoblastoma cell line (Y79) may represent an additional example of α_{2C}-adrenoceptor. Br. J. Pharmacol. 107, 222–225.

Hieble, J.P., Bondinell, W.E., Ruffolo, R.R. Jr., 1995. α- and β-Adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. J. Med. Chem. 38, 3415–3444.

Kazmi, S.M.I., Mishra, R.K., 1989. Identification of α_2-adrenergic receptor sites in human retinoblastoma (Y-79) and neuroblastoma (SH-SY5Y) cells. Biochem. Biophys. Res. Commun. 158, 921–928.

Kobilka, B.K., Matsui, H., Kobilka, T.S., Yang-Feng, T.L., Francke, U., Caron, M.G., Lefkowitz, R.J., Regan, J.W., 1987. Cloning, sequencing, and expression of the gene coding for the human platelet α_2-adrenergic receptor. Science 238, 650–656.

Lameh, J., Eiger, S., Sadée, W., 1992. Interaction among μ-opioid receptors and α_2-adrenoceptors on SH-SY5Y human neuroblastoma cells. Eur. J. Pharmacol. 227, 19–24.

Lomasney, J.W., Lorenz, W., Allen, L.F., King, K., Regan, J.W., Yang-Feng, T.L., Caron, M.G., Lefkowitz, R.J., 1990. Expansion of the α_2-adrenergic receptor family: cloning and characterization of a human α_2-adrenergic receptor subtype, the gene for which is located on chromosome 2. Proc. Natl. Acad. Sci. USA 87, 5094–5098.

MacDonald, E., Kobilka, B.K., Scheinin, M., 1997. Gene targeting—homing in on α_2-adrenoceptor subtype function. Trends Pharmacol. Sci. 18, 211–219.

Marjamäki, A., Luomala, K., Ala-Uotila, S., Scheinin, M., 1993. Use of recombinant α_2-adrenoceptors to characterize subtype selectivity of antagonist binding. Eur. J. Pharmacol. 246, 219–226.

Murphy, T.J., Bylund, D.B., 1988. Characterization of alpha-2 adrenergic receptors in the OK cell, an opossum kidney cell line. J. Pharmacol. Exp. Ther. 244, 571–578.

Pohjanoksa, K., Jansson, C.C., Luomala, K., Marjamäki, A., Savola, J.M., Scheinin, M., 1997. α_2-Adrenoceptor regulation of adenylyl

cyclase in CHO cells: dependence on receptor density, receptor subtype and current activity of adenylyl cyclase. Eur. J. Pharmacol. 335, 53–63.

Regan, J.W., Kobilka, T.S., Yang-Feng, T.L., Caron, M.G., Lefkowitz, R.J., Kobilka, B.K., 1988. Cloning and expression of a human kidney cDNA for an α_2-adrenergic receptor subtype. Proc. Natl. Acad. Sci. USA 85, 6301–6305.

Schaak, S., Cayla, C., Blaise, R., Quinchon, F., Paris, H., 1997. HepG2 and SK-N-MC: two human models to study alpha-2 adrenergic receptors of the alpha-2C subtype. J. Pharmacol. Exp. Ther. 281, 983–991.

Schoeffter, P., Bobirnac, I., Boddeke, E., Hoyer, D., 1997. Inhibition of cAMP accumulation via recombinant human serotonin 5-HT_{1A} receptors: considerations on receptor-effector coupling across systems. Neuropharmacology 36, 429–437.

Trendelenburg, A.U., Limberger, N., Rump, L.C., 1994. α_2-Adrenergic receptors of the α_{2C} subtype mediate inhibition of noradrenaline release in human kidney cortex. Mol. Pharmacol. 45, 1168–1176.

Uhlen, S., Porter, A.C., Neubig, R.R., 1994. The novel alpha-2 adrenergic radioligand [^{3}H]-MK912 is alpha-2C selective among human alpha-2A, alpha-2B and alpha-2C adrenoceptors. J. Pharmacol. Exp. Ther. 271, 1558–1565.

West, R.E., Moss, J., Vaughan, M., Liu, T., Liu, T.Y., 1985. Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cysteine 347 is the ADP-ribose acceptor site. J. Biol. Chem. 260, 14428–14430.