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α_2-Adrenoceptor regulation of adenylyl cyclase in CHO cells: dependence on receptor density, receptor subtype and current activity of adenylyl cyclase

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Abstract

Chinese hamster ovary (CHO) cells stably transfected to express different densities of the human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor subtypes, were used to characterize the regulation of adenylyl cyclase activity by α_2-adrenoceptor agonists. In isolated cell membranes, activation of α_{2A}- and α_{2C}-adrenoceptors did not affect basal enzyme activity, but activation of α_{2B}-adrenoceptors stimulated adenylyl cyclase activity. The extent of stimulation was dependent on the receptor density and was insensitive to pertussis toxin treatment. In the presence of 10 μM forskolin all three receptor subtypes mediated inhibition of adenylyl cyclase activity in a pertussis toxin-sensitive manner. In experiments performed with intact cells the same pattern could be seen: the basal production of cAMP was not affected when α_{2C}-adrenoceptors were activated, but activated α_{2B}-adrenoceptors mediated stimulation of cAMP production. In the presence of forskolin, both receptor subtypes mediated inhibition of cAMP production. Our results suggest that α_{2B}-adrenoceptors are coupled to both G_i and G_s proteins. The signal transduction pathway to which the receptor is coupled is not dependent on receptor density, but its effect on adenylyl cyclase regulation is dependent on the current activity of adenylyl cyclase. The results also suggest that the α_{2A}- and α_{2C}-subtypes are preferentially coupled to G_i and transduce only inhibition of adenylyl cyclase activity in transfected CHO cells. At low densities of α_{2C}-adrenoceptors, clonidine was a partial agonist, but in clones expressing high levels of α_{2C}-adrenoceptors, clonidine acted as a full agonist by inhibiting cAMP accumulation with the same efficacy as (−)-noradrenaline. This demonstrates that receptor reserve can mask partial agonist activity. © 1997 Elsevier Science B.V.

Keywords: α_2-Adrenoceptor subtype; CHO cell; [^{3}H]RX821002 binding; Adenylyl cyclase; cAMP

1. Introduction

The α_2-adrenoceptors (α_{2A}, α_{2B} and α_{2C}) are members of the G-protein coupled receptor superfamily. They mediate both inhibitory and stimulatory effects at the second messenger level. The classical inhibitory effects of α_2-adrenoceptors are transduced by the pertussis toxin sensitive G-proteins G_i and G_o, and include inhibition of adenylyl cyclase activity, reduced voltage-gated Ca^{2+} channel activity (Holz et al., 1986) and neuronal hyperpolarization through increased K^+ conductance (Aghajanian and VanderMaelen, 1982). Stimulatory effects have been less well characterized. They include activation of phospholipases A_2, C and D (Cotecchia et al., 1990; Jones et al., 1991; MacNulty et al., 1992), stimulation of adenylyl cyclase activity (Eason et al., 1992) and mobilization of intracellular Ca^{2+} (Michel et al., 1989; Enkvist et al., 1996).

Abbreviations: CHO, Chinese hamster ovary cells; PC12, rat pheochromocytoma cells; JEG-3, human choriocarcinoma cells; Sf-9, *Spodoptera frugiperda* insect ovary cell; S115, mouse mammary tumor cell; NG108-15, mouse neuroblastoma × rat glioma hybrid cell; DDT_1MF-2, hamster smooth muscle cells; NIH-3T3, mouse fibroblast cells; HT-29, human colonic adenocarcinoma cells; HEL, human erythroleukemia cells

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The specific coupling characteristics of each α_2-adrenoceptor subtype have not been elucidated in detail. Studies on recombinant α_2-adrenoceptors expressed in different cell lines have demonstrated that each receptor is capable of coupling to several signal transduction systems (Regan and Cotecchia, 1992). It has been shown that all three subtypes have the potential to couple physically and functionally not only to G_i but also to G_s in membranes from CHO cells (Eason et al., 1992). In studies performed on intact cells, the α_{2B}-subtype mediates stimulatory effects on adenylyl cyclase in PC12, JEG-3 and Sf9 cells (Duzic and Lanier, 1992; Pepperl and Regan, 1993; Jansson et al., 1995). In addition, when expressed in S115 cells, the α_{2B}-subtype mediates stimulation of adenylyl cyclase after treatment of the cells with pertussis toxin (to block G_i-mediated effects), an effect not seen with the other two subtypes (Jansson et al., 1994a). This stimulatory effect on adenylyl cyclase by the α_{2B}-subtype is not seen in all types of cultured cells, because activation of this receptor in NG108-15, DDT_1MF-2, NIH-3T3 and astroglia cells inhibits adenylyl cyclase (Sabol and Nirenberg, 1979; Duzic and Lanier, 1992; Enkvist et al., 1996). Activation of α_{2A}- adrenoceptors in CHO, PC12 and JEG-3 cells has been reported to result in biphasic responses in cAMP production. At low concentrations (<100 nM) of potent agonists, adenylyl cyclase activity is inhibited, and at concentrations above 100 nM adenylyl cyclase is activated (Fraser et al., 1989; Jones et al., 1991; Duzic and Lanier, 1992; Pepperl and Regan, 1993). In many other cell types, e.g. HT-29, DDT_1MF-2, NIH-3T3, Chinese hamster lung fibroblasts, S115, Sf9 and HEL cells, only inhibition of adenylyl cyclase has been obtained by activation of α_{2A}-adrenoceptors (Turner et al., 1985; Cotecchia et al., 1990; Duzic and Lanier, 1992; Jansson et al., 1994a,b, 1995; unpublished data). The α_{2C}-adrenoceptor has been expressed in many different cell types, and activation of this receptor in intact cells has always resulted in inhibitory effects on adenylyl cyclase (Cotecchia et al., 1990; Oker-Blom et al., 1993; Blaxall et al., 1994; Jansson et al., 1994a,b, 1995). Stimulation of adenylyl cyclase activity through this receptor has, however, been reported in membranes prepared from pertussis toxin treated CHO cells (Eason et al., 1992).

The mechanism mediating the stimulatory effect of α_{2B}-adrenoceptors on adenylyl cyclase in intact cells has not been well characterized. In PC12 cells expressing the α_{2B}-subtype the stimulatory effect was blocked when intracellular Ca^{2+} was chelated with bis-(*o*-aminophenoxy)-ethene-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (Duzic and Lanier, 1992). In intact Sf9 cells the stimulatory effect mediated by the α_{2B}-subtype was blocked by cholera toxin, indicating the involvement of G_s-proteins (Jansson et al., 1995). Another possible transduction pathway for stimulation of adenylyl cyclase is through the $\beta\gamma$ subunits released from activated G_i-proteins, as has been suggested for muscarinic M_3 receptors (Baumgold, 1992).

The aim of this study was to further characterize the coupling of the three α_2-adrenoceptor subtypes to regulation of adenylyl cyclase activity in Chinese hamster ovary (CHO) cells. This was considered important because of two reasons. First, this well-characterized system allows the separate and controlled expression of the human α_2-adrenoceptor subtypes without any influence from endogenous α_2-adrenoceptors. Identification of distinct coupling patterns for the three closely related receptors may lead to new insights into drug discovery and development. Second, we wanted to test the influence of receptor density on agonist responses, since transfected CHO cells are widely used for the characterization of new α_2-adrenoceptor agonists. We predicted that a high receptor density would interfere with the discrimination between full and partial agonists. The study was carried out using both intact cells and isolated cell membranes. Membrane preparations were used in order to investigate the direct coupling between receptors, G-proteins and adenylyl cyclases, without interference of soluble intracellular modulators and effectors.

2. Materials and methods

2.1. Materials

[^{3}H]RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline) (48 Ci/mmol), [^{3}H]adenine, [^{3}H]cAMP and [^{14}C]cAMP were from Amersham (Amersham, UK). [^{32}P]ATP was from DuPont-NEN (Dreieich, Germany). Clonidine, forskolin, 5′-guanylylimidodiphosphate (Gpp(NH)p), Geneticin® (G418), isobutylmethylxanthine (IBMX), imidazole, (−)-noradrenaline, oxymetazoline, propranolol and quinacrine were from Sigma (St. Louis, MO, USA). UK14,304 (5-bromo-6-[2-imidazoline-2-ylamino]quinoxaline), dexmedetomidine and levomedetomidine were gifts from Orion Corporation Orion-Pharma (Turku, Finland).

2.2. Methods

2.2.1. Construction of expression vectors

Expression vectors based on pMAMneo (Clontech, Palo Alto, CA, USA), containing a neomycin (G418) resistance gene for the selection of stably transfected cell clones, were constructed using standard methods. The cDNAs encoding the human α_2-adrenoceptor subtypes were gifts from Dr. R.J. Lefkowitz (Kobilka et al., 1987; Regan et al., 1988; Lomasney et al., 1990). All expression vectors contained an extra 337 bp RSV LTR (Rous sarcoma virus long terminal repeat) transcription control element isolated from the plasmid pBC12I (Cullen, 1987). The addition of this promoter element to pMAMneo has in our hands resulted in increased expression levels of recombinant receptors in CHO cells.

2.2.2. Transfection, selection and cell culture

Adherent CHO cells (American Type Culture Collection, Rockville, MD, USA) were cultured in α-MEM (Modified Eagle Medium) supplemented with 2 mM glutamine, 20 mM $NaHCO_3$, 5% heat-inactivated fetal calf serum (Gibco, Paisley, UK), penicillin (50 U/ml) and streptomycin (50 μg/ml). Cells were grown in 5% CO_2 at 37°C. The expression constructs were transfected into CHO cells with the Lipofectin® reagent kit (Gibco, Gaithersburg, MD, USA). For each transfection 3 μg plasmid DNA was used per 5×10^4 cells. Ten to fifty G418 (750 μg/ml) resistant clones from each transfection were selected and examined for their ability to bind the α_2-adrenoceptor antagonist [^{3}H]RX821002. The clones chosen for further experiments were subsequently maintained in 250 μg/ml G418. For some experiments, cells were grown for 24 h in serum-free medium supplemented with 500 ng/ml pertussis toxin.

2.2.3. Preparation of cell membranes

Cells were detached from culture flasks with 0.01% trypsin/0.02% K-EDTA, centrifuged at $1500 \times g$ for 5 min at 4°C, and washed once with chilled phosphate-buffered saline (PBS). The pellet was suspended in ice-cold lysis buffer (5 mM Tris–HCl, 5 mM EDTA, pH 7.4) and homogenized in a Potter S homogenizer (B. Braun, Melsungen), 10–15 strokes at 1000 rpm on ice. The cell homogenate was centrifuged at $200 \times g$ for 10 min at 4°C. The supernatant was further centrifuged at $47\,000 \times g$ for 30 min at 4°C. The resulting pellet was washed once and then resuspended in the buffer used for determination of adenylyl cyclase activity (40 mM Hepes, 0.8 mM EDTA, 1.6 mM $MgCl_2$, 100 mM NaCl, pH 7.4).

2.2.4. Ligand binding

Ligand binding assays were performed in K^+-phosphate buffer as previously described (Halme et al., 1995). In saturation studies, whole cell homogenates containing 30–50 μg of protein were incubated with [^{3}H]RX821002 (0.06–8 nM) in a final volume of 0.25 ml K^+-phosphate buffer at 25°C for 20 min. Non-specific binding was determined by including 10 μM (−)-adrenaline in parallel assays. Competition studies were performed using [^{3}H]RX821002 concentrations close to the K_d for each receptor subtype and 13–15 concentrations of the competitors. The GTP dependence of agonist binding was investigated with the stable GTP analog Gpp(NH)p; a Gpp(NH)p concentration of 10 μM was selected for this study (Marjamäki et al., 1992). Bound radioactivity was separated by filtration on Whatman GF/B filter strips, using a Brandel cell harvester (Model M-48R, Gaithersburg, MD) and two 5 ml washes with ice-cold K^+-phosphate buffer. Radioactivity on the filters was determined by liquid scintillation counting (Wallac 1410, Wallac, Turku) in OptiPhase 'HiSafe' III (Wallac). Protein concentrations were determined according to the method of Bradford (1976), using bovine serum albumin as reference.

2.2.5. Measurement of adenylyl cyclase activity

Fresh membranes (5–10 μg) were incubated in a reaction mixture (50 μl) consisting of 0.12 mM ATP, 3 μM GTP, 0.1 mM cAMP, 2.7 mM phosphoenolpyruvate, 20 U/ml myokinase, 4 U/ml pyruvate kinase, 0.5 mg/ml bovine serum albumin, 0.12 mM IBMX, and [α-^{32}P]ATP ($1–2 \times 10^6$ dpm/tube). The effect of different concentrations (1 nM–100 μM) of different α_2-ligands were measured both in the absence and in the presence of 10 μM forskolin. The reactions (10 min at 37°C) were terminated by the addition of 1 ml of 0.4 mM ATP, 0.3 mM cAMP and [^{3}H]cAMP (about 70 000 dpm/tube). [^{32}P]cAMP was isolated by sequential chromatography over Dowex and alumina columns (Salomon et al., 1974) and normalized to the recovery of [^{3}H]cAMP tracer (generally 70–80%). Radioactivity was determined by liquid scintillation counting as described above. The adenylyl cyclase activity was expressed as pmol ATP converted to cAMP/mg/min.

2.2.6. Measurement of cAMP production

The growth medium of confluent cultures was replaced with serum-free fresh medium supplemented with 5 μCi/ml of [^{3}H]adenine. After incubation for 2 h the cells were detached with EDTA (0.02%) and washed once in physiological assay buffer (137 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 20 mM TES (2-{(3-hydroxyl-1,1-bis(hydroxymethyl)ethyl}ethane sulphonate), 10 mM glucose and 1 mM $CaCl_2$ adjusted to pH 7.4). Thereafter the cells were suspended in the same buffer and divided into aliquots of about 1×10^6 cells in 0.8 ml buffer. The cells were preincubated with 0.5 mM IBMX (a non-specific phosphodiesterase inhibitor), 100 μM propranolol (a β-adrenoceptor antagonist) and 150 μM quinacrine (a phospholipase A_2 inhibitor) for 10 min at 37°C. Forskolin (5 μM) and agonists in different concentrations (1 nM–100 μM) were added. After 10 min the cells were centrifuged for 1 min at $10\,000 \times g$, the medium was immediately removed and the reaction was terminated by resuspension of the cells in 1 ml 0.33 M perchloric acid, containing about 1600 dpm [^{14}C]cAMP. The extent of conversion of [^{3}H]ATP to [^{3}H]cAMP was determined by sequential Dowex/alumina ion exchange chromatography to isolate cAMP (Salomon et al., 1974). Conversion to [^{3}H]cAMP was expressed as percentage of total cell-associated tritium and was normalized to the recovery of [^{14}C]cAMP tracer (generally 70%). Radioactivity was determined by liquid scintillation counting as described above.

2.2.7. Data analysis

All data were analyzed using GraphPAD Prism programs (GraphPAD Software, San Diego, CA). Statistical analysis was carried out by Student's *t*-test with two-tailed

Table 1
Affinities of four different α_2-adrenoceptor ligands for the α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors, separately expressed in CHO cells at densities close to 1 pmol/mg protein

Ligand	App. K_i (nM)	Slope	K_{iH} (nM)	K_{iL} (nM)	% H	App. K_i + Gpp(NH)p (nM)	Slope	App. K_i + Gpp(NH)p/ App. Ki	K_{iL} / K_{iH}
A: CHO, α_{2A}-E47									
Noradrenaline	277 ± 145	0.19 ± 0.14	11.1 ± 13.5	2428 ± 734	44 ± 8	1186 ± 64.0	0.59 ± 0.06	4.3	219
Dexmedetomidine	2.20 ± 0.25	0.56 ± 0,03	0.13 ± 0,16	7.00 ± 3.70	34 ± 12	5.93 ± 0.32	0.85 ± 0.11	2.7	56
Levomedetomidine	42.9 ± 7.30	0.92 ± 0.16	5.09 ± 3.9	65.8 ± 8.90	28 ± 2	36.2 ± 2.20	0.94 ± 0.07	0.8	13
Clonidine	17.2 ± 1.54	0.55 ± 0.11	2.19 ± 2.06	77.2 ± 29.4	50 ± 6	62.7 ± 3.38	0.83 ± 0.05	3.7	35
B: CHO, α_{2B}-2									
Noradrenaline	500 ± 206	0.64 ± 0.08	1.51 ± 0.57	805 ± 364	21 ± 2	631 ± 127	0.73 ± 0.05	1.3	532
Dexmedetomidine	3.33 ± 1.41	0.69 ± 0.08	0.43 ± 0.44	7.26 ± 3.25	28 ± 4	5.57 ± 0.99	0.72 ± 0.08	1.7	17
Levomedetomidine	91.7 ± 54.2	0.79 ± 0.08	24.3 ± 14.4	223 ± 66.2	46 ± 13	122 ± 3.60	0.82 ± 0.04	1.3	9
Clonidine	56.0 ± 12.6	0.92 ± 0.16	1.00 ± 0.27	64.2 ± 13.8	21 ± 2	65.4 ± 13.1	0.89 ± 0.15	1.2	64
C: CHO, α_{2C}-C1									
Noradrenaline	256 ± 102	0.59 ± 0.12	1.64 ± 0.98	408 ± 50.0	29 ± 6	516 ± 114	0.65 ± 0.21	2.0	250
Dexmedetomidine	2.97 ± 1.78	0.69 ± 0.13	1.02 ± 0.80	18.6 ± 12.6	52 ± 10	6.15 ± 0.30	0.92 ± 0.07	2.1	18
Levomedetomidine	40.9 ± 7.60	0.80 ± 0.04	8.94 ± 3.25	58.4 ± 32.2	39 ± 0.9	29.3 ± 0.36	0.81 ± 0.07	0.7	6.5
Clonidine	96.9 ± 8.69	0.72 ± 0.09	5.56 ± 1.53	180 ± 52.1	22 ± 5	184 ± 30.3	0.79 ± 0.20	1.9	32

The competition binding assays were performed with cell homogenates and [^{3}H]RX821002 as radioligand. One clone representing each subtype was used, α_{2A}-E47, α_{2B}-2 and α_{2C}-C1. Apparent (App.) K_i is the binding constant for a one-site model. Slope indicates the Hill coefficient. K_{iH} and K_{iL} are inhibition constants for the high- and low-affinity sites in a two-site model. % H is the percentage of binding sites in the high-affinity state. The values are means ± S.E. from three independent experiments performed in triplicate.

probabilities or one-way analysis of variance (ANOVA) followed by Dunnett's test. P values lower than 0.05 were considered to be statistically significant. The results are expressed as mean values ± S.E.

3. Results

3.1. Ligand binding experiments

Chinese hamster ovary (CHO) cells were stably transfected to express different densities of the human α_{2A}-, α_{2B}- and α_{2C}-adrenoceptor subtypes. The receptor densities were determined in saturation binding experiments using [^{3}H]RX821002 as radioligand. The receptor densities of the cell clones used in this study and the corresponding K_d of the radioligand are presented in Table 2. No specific binding of [^{3}H]RX821002 was observed in non-transfected CHO cells.

Competition binding experiments were performed using one clone of each receptor subtype (α_{2A}-E47, α_{2B}-2 and α_{2C}-C1), all expressing about 1 pmol receptors/mg total cellular protein. Oxymetazoline competed with [^{3}H]RX821002 with the expected order of potency at the three subtypes (K_i values: $\alpha_{2A} = 8.0 \pm 1.4$ nM; $\alpha_{2B} = 1040 \pm 300$ nM; $\alpha_{2C} = 89 \pm 8.0$ nM). The agonists (−)-noradrenaline, dexmedetomidine, levomedetomidine and clonidine also inhibited binding of [^{3}H]RX821002. The competition curves for (−)-noradrenaline, dexmedetomidine and clonidine were best modelled by two-site fits (Hill slopes < 1) for all subtypes (Table 1). The addition of 10 μM Gpp(NH)p induced rightward shifts in the high-affinity components of the curves, and the results were now best modelled by one-site fits (Hill slopes closer to 1). The competition curves for levomedetomidine were equally well modelled by one-site and two-site fits for all subtypes. When comparing the apparent binding affinity constants (App. K_i) derived from one-site models for the different ligands, dexmedetomidine showed highest affinity for all three subtypes (2–3 nM). Levomedetomidine and clonidine had intermediate affinity (40–90 nM and 17–100 nM, respectively) and (−)-noradrenaline had lowest affinity for all subtypes (250–500 nM). None of the ligands showed marked subtype selectivity. The Gpp(NH)p-induced reductions in agonist affinity and the ratios between the low- and high-affinity binding constants (K_{iL}/K_{iH}) are presented numerically in Table 1. No clear differences could be seen in the Gpp(NH)p-induced affinity shifts of (−)-noradrenaline, dexmedetomidine and clonidine. In contrast, when comparing the K_{iL}/K_{iH}-ratios obtained for these ligands, clear differences were evident. (−)-Noradrenaline had clearly higher K_{iL}/K_{iH}-ratios than the other ligands for all receptor subtypes (200–500), dexmedetomidine and clonidine had intermediate ratios for all subtypes (10–60), and levomedetomidine had the lowest ratio for all subtypes (6–13). Dexmedetomidine was the only ligand to show some subtype preference by having a 3 times higher K_{iL}/K_{iH}-ratio for the α_{2A}-subtype than for the other two subtypes.

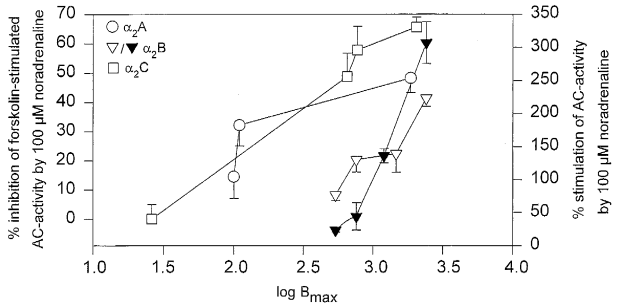

Fig. 1. The maximal inhibitory effect of (−)-noradrenaline (100 μM) on forskolin-stimulated adenylyl cyclase (AC) activity in different clones expressing α_{2A}-, α_{2B}- or α_{2C}-adrenoceptors, and the maximal stimulatory effect of (−)-noradrenaline (100 μM) (▼) in the absence of forskolin in the clones expressing α_{2B}-adrenoceptors are plotted against the receptor density (B_{max}). The values are means ± S.E. from 3–6 experiments performed in triplicate.

3.2. Adenylyl cyclase activity

The basal adenylyl cyclase activity of the cell clones was 4–12 pmol/mg/min and was not influenced by recombinant α_2-adrenoceptor expression (data not shown). Addition of (−)-noradrenaline, dexmedetomidine, clonidine or levomedetomidine (1 nM–100 μM) to membranes from non-transfected cells or to membrane preparations from cells transfected to express different densities of the α_{2A}- or α_{2C}-adrenoceptor subtypes did not result in any significant changes in adenylyl cyclase activity (data not shown). In contrast, in membranes expressing the α_{2B}-subtype, (−)-noradrenaline and dexmedetomidine clearly stimulated adenylyl cyclase activity (Table 2). The efficacy of agonist stimulation was dependent on receptor density in a log–linear fashion (Fig. 1). In the clone expressing the lowest density of α_{2B}-adrenoceptors (α_{2B}-3), a statistically significant stimulation could only be seen with (−)-noradrenaline (23%) (Table 2). In the clones expressing intermediate densities (α_{2B}-2 and α_{2B}-10), dexmedetomidine and clonidine also stimulated adenylyl cyclase activity. In the clone expressing the highest receptor density (α_{2B}-6), adenylyl cyclase was stimulated by all four ligands, and the rank order of efficacy was (−)-noradrenaline (307%) > dexmedetomidine (254%) > clonidine (48%) > levomedetomidine (28%). The stimulatory effects were monophasically concentration-dependent, and dexmedetomidine stimulated adenylyl cyclase activity with a clearly higher sensitivity ($EC_{50} = 35$ nM) than the other agonists ($EC_{50} = 1000–3000$ nM) (Table 2).

Addition of 10 μM forskolin (10 min) resulted in 5- to 10-fold increases in adenylyl cyclase activity (data not shown). Forskolin-stimulated enzyme activity was inhibited by (−)-noradrenaline, dexmedetomidine, levomedeto-

Table 2

Characterization of adenylyl cyclase activity in membranes from CHO cells

CHO-α_{2A}	α_{2A}-E27 (B_{max} = 0.10 ± 0.03 pmol/mg, K_d = 0.38 ± 0.14 nM)		α_{2A}-E30 (B_{max} = 0.11 ± 0.01 pmol/mg, K_d = 0.26 ± 0.10 nM)		α_{2A}-E47 (B_{max} = 1.88 ± 0.40 pmol/mg, K_d = 0.72 ± 0.06 nM)			
	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)		
Ligand + forskolin:								
Noradrenaline	−14.6 ± 7.4 (ns)	—	−32.2 ± 7.1	1652 ± 394	−48.3 ± 5.0	294 ± 140		
Dexmedetomidine	−12.8 ± 1.5 (ns)	—	−21.7 ± 2.6	6.3 ± 5.2	−43.2 ± 4.2	3.6 ± 2.1		
Levomedetomidine	−9.0 ± 8.9 (ns)	—	−2.1 ± 4.5 (ns)	—	−6.9 ± 4.6 (ns)	—		
Clonidine	−5.1 ± 5.2 (ns)	—	−23.2 ± 1.7	798 ± 1036	−44.6 ± 4.0	33.8 ± 9.6		
CHO-α_{2B}	α_{2B}-3 (B_{max} = 0.54 ± 0.18 pmol/mg, K_d = 2.20 ± 0.08 nM)		α_{2B}-10 (B_{max} = 0.76 ± 0.32 pmol/mg, K_d = 2.28 ± 0.40 nM)		α_{2B}-2 (B_{max} = 1.47 ± 0.25 pmol/mg, K_d = 3.43 ± 0.12 nM)		α_{2B}-6 (B_{max} = 2.40 ± 0.65 pmol/mg, K_d = 2.07 ± 0.31 nM	
	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)
Ligand − forskolin:								
Noradrenaline	23.4 ± 2.8	2846 ± 2686	44.2 ± 20.8	3566 ± 2078	136 ± 10.6	2830 ± 1904	307 ± 31.4	2844 ± 457
Dexmedetomidine	8.2 ± 11.3 (ns)	—	35.3 ± 14.6	48.7 ± 38.8	88.2 ± 22.0	32.1 ± 26.3	254 ± 14.5	35.3 ± 11.9
Levomedetomidine	−7.5 ± 8.6 (ns)	—	−17.4 ± 11.6 (ns)	—	6.6 ± 9.3 (ns)	—	28.3 ± 6.2	1047 ± 116
Clonidine	7.4 ± 8.9 (ns)	—	25.4 ± 10.6 (ns)	—	30.3 ± 19.9 (ns)	—	48.2 ± 11.5	982 ± 78.0
Ligand + forskolin:								
Noradrenaline	−8.32 ± 1.86 (ns)	—	−20.4 ± 4.2	447 ± 80.0	−22.4 ± 6.3	178 ± 99.6	−41.4 ± 2.8	85.7 ± 6.9
Dexmedetomidine	−9.66 ± 0.76 (ns)	—	−13.7 ± 5.1	5.25 ± 3.4	−18.5 ± 2.0	0.69 ± 0.3	−45.27 ± 2.2	0.4 ± 0.2
Levomedetomidine	−13.78 ± 5.42 (ns)	—	−8.66 ± 5.4	—	−19.9 ± 2.5	170 ± 131	−29.86 ± 5.1	542 ± 470
Clonidine	−8.24 ± 8.41 (ns)	—	−14.5 ± 5.4	294 ± 472	−15.0 ± 9.1	9.33 ± 9.6	−44.1 ± 4.4	117 ± 60.9
CHO-α_{2C}	α_{2C}-L2 (B_{max} = 0.03 ± 0.01 pmol/mg, K_d = 0.72 ± 0.40 nM)		α_{2C}-R1-6 (B_{max} = 0.65 ± 0.12 pmol/mg, K_d = 0.98 ± 0.13 nM)		α_{2C}-C1 (B_{max} = 0.77 ± 0.25 pmol/mg, K_d = 0.83 ± 0.11 nM)		α_{2C}-L3 (B_{max} = 2.04 ± 0.42 pmol/mg, K_d = 0.70 ± 0.12 nM	
	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)
Ligand + forskolin:								
Noradrenaline	0.1 ± 5.0 (ns)	—	−48.8 ± 8.0	406 ± 184	−57.8 ± 8.1	348 ± 39.5	−65.6 ± 3.4	243 ± 194
Dexmedetomidine	−28.6 ± 23.8 (ns)	—	−45.1 ± 8.6	5.0 ± 3.2	−61.5 ± 3.4	1.4 ± 1.27	−62.2 ± 5.2	6.6 ± 3.5
Levomedetomidine	−4.3 ± 5.5 (ns)	—	−6.2 ± 3.9 (ns)	—	−13.6 ± 8.3 (ns)	—	−10.6 ± 7.8	497 ± 379
Clonidine	−8.8 ± 12.0 (ns)	—	−23.4 ± 4.9	141 ± 92.2	−47.4 ± 8.8	47.2 ± 10.5	−40.7 ± 7.8	206 ± 145

The E_{max} values for the different drugs in cell clones expressing α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors indicate the maximal % changes from the enzyme activity in the absence of agonists. Negative values indicate % inhibition and positive values indicate % stimulation. EC_{50} is the concentration of each drug causing 50% of the maximal effect. + or − forskolin indicates presence or absence of 10 μM forskolin. Specific binding of [^{3}H]RX821002 was measured in whole cell homogenates, and the B_{max} and K_d indicate receptor density and affinity. The values are means ± S.E. from 3–6 experiments performed in duplicate. The statistical significance of the drug effects was calculated by ANOVA and Dunnett's test, using the original pmol/mg/min values. ns, not significant.

midine and clonidine acting through all three receptors (Table 2). The highest concentrations (100 μM) of dexmedetomidine and levomedetomidine inhibited forskolin-stimulated adenylyl cyclase activity also in non-transfected cells, by approximately 20%, by an unknown mechanism. Therefore all dexmedetomidine and levomedetomidine results were calculated excluding the highest drug concentration. The inhibitory effects of agonists on forskolin-stimulated adenylyl cyclase activity were most pronounced in the clones expressing the highest receptor densities. In the α_{2A}-E47 clone the maximal inhibitory effect was approximately 45% for (−)-noradrenaline, dexmedetomidine and clonidine and 7% for levomedetomidine. The forskolin-stimulated activities were inhibited by 40% by (−)-noradrenaline, dexmedetomidine and clonidine, and by 30% by levomedetomidine in the α_{2B}-6 clone. The maximal inhibitory effect in the α_{2C}-L3 clone was approximately 65% for (−)-noradrenaline and dexmedetomidine, 41% for clonidine and 11% for levomedetomidine (Table 2).

The inhibitory effects, in the clones expressing highest receptor densities, were induced with highest sensitivity by dexmedetomidine for all three receptor subtypes (EC_{50} = 0.2–7 nM). (−)-Noradrenaline and clonidine inhibited adenylyl cyclase with roughly similar sensitivity for all subtypes (30–300 nM). Dexmedetomidine showed subtype selectivity by inducing inhibition through α_{2B}-adrenoceptors (in the α_{2B}-6 clone) with 25 times higher sensitivity compared to that for the other two receptor subtypes (in the α_{2A}-E47 and α_{2C}-L3 clones). The inhibitory effects were transduced with slightly lower sensitivity in the clones expressing lower densities of the receptors.

In Fig. 1 the maximal effects of (−)-noradrenaline are plotted against receptor density in the different clones. It can be noted that about 2 pmol/mg of the α_{2B}-adrenoceptors was needed in order to obtain 40% inhibition of forskolin-stimulated adenylyl cyclase activity. Similar inhibition was mediated by about 0.3 pmol/mg of the α_{2A}- and α_{2C}-subtypes.

Pretreatment with pertussis toxin resulted in complete block of the inhibitory effects of the agonist at all subtypes (Table 3, data not shown for the α_{2A}- and α_{2C}-subtypes). In addition, a further stimulation of forskolin-stimulated adenylyl cyclase activity could be seen in pertussis toxin-treated cells expressing the α_{2B}-subtype. This stimulatory effect, which was seen with all tested agonists except levomedetomidine, was most pronounced in the clone expressing the highest receptor density (α_{2B}-6), and no stimulation could be seen in the α_{2B}-10 clone with the lowest receptor density. The rank order of efficacy for stimulation of adenylyl cyclase in the presence of forskolin was the same as that observed in cells not pretreated with pertussis toxin and in the absence of forskolin ((−)-noradrenaline (72%) > dexmedetomidine (66%) > clonidine (16%)). The stimulatory effects of the ligands on basal adenylyl cyclase activity (in the absence of forskolin) were not significantly affected by pertussis toxin treatment.

3.3. cAMP production

The conversion of [^{3}H]ATP to [^{3}H]cAMP was monitored to assess the coupling of α_2-adrenoceptors to regulation of cAMP production in intact CHO cells. These experiments were performed on the clones α_{2B}-3, α_{2B}-6, α_{2C}-C1, α_{2C}-L2, α_{2C}-L3 and α_{2C}-R1-6 in order to compare two receptor subtypes, α_{2B} and α_{2C}, which had shown clearly different responses in the adenylyl cyclase assay performed with isolated cell membranes. The non-stimulated conversion of [^{3}H]ATP to [^{3}H]cAMP was similar in non-transfected cells and in transfected cells expressing α_2-adrenoceptor subtypes (0.02–0.09% in 10 min) (data not shown). Addition of 100 μM of the ligands (−)-noradrenaline, dexmedetomidine, levomedetomidine or clonidine did not result in any significant changes in the

Table 3
Stimulation of adenylyl cyclase activity mediated by α_{2B}-adrenoceptors after pertussis toxin treatment

Pertussis toxin	α_{2B}-10		α_{2B}-2		α_{2B}-6	
	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)	E_{max}	EC_{50} (nM)
Ligand − forskolin						
Noradrenaline	57.2 ± 19.8	3412 ± 1030	113 ± 38.6	2549 ± 356	268 ± 49.9	3396 ± 1141
Dexmedetomidine	38.3 ± 3.4	84.6 ± 26.2	122 ± 21.4	18.3 ± 3.5	185 ± 6.0	30.1 ± 9.7
Levomedetomidine	4.2 ± 7.2 (ns)	—	−4.6 ± 5.8 (ns)	—	13.7 ± 6.8 (ns)	—
Clonidine	9.1 ± 4.1 (ns)	—	13.7 ± 3.4 (ns)	—	53.8 ± 9.2	218 ± 149
Ligand + forskolin						
Noradrenaline	−0.8 ± 14.2 (ns)	—	20.8 ± 3.5	2373 ± 1686	71.9 ± 5.2	2419 ± 582
Dexmedetomidine	11.3 ± 5.3 (ns)	—	37.5 ± 12.2	20.5 ± 4.7	65.6 ± 4.1	45.8 ± 24.2
Levomedetomidine	8.6 ± 0.6 (ns)	—	1.5 ± 6.7 (ns)	—	2.0 ± 2.7 (ns)	—
Clonidine	4.3 ± 4.2 (ns)	—	8.2 ± 3.5 (ns)	—	16.0 ± 3.1	55.1 ± 51.2

CHO cells were incubated for 24 h with 500 ng/ml pertussis toxin. Membranes were prepared and the ability of agonists to induce changes in adenylyl cyclase activity was measured. The maximal responses (E_{max}) are expressed as % increases over the enzyme activity in the absence of agonists. EC_{50} is the concentration of each drug causing 50% of the maximal effect. + or − forskolin indicates presence or absence of 10 μM forskolin. The values are means ± S.E. from 3–6 experiments performed in duplicate. The statistical significance as in Table 2.

cAMP production in non-transfected cells or in cells expressing α_{2C}-adrenoceptors (data not shown). Addition of 100 μM of (−)-noradrenaline or dexmedetomidine to the α_{2B}-3 clone resulted in small non-significant increases in cAMP production over the basal level (32% and 42%, respectively) (Table 4). The corresponding increases induced by the two ligands were clearly greater in the α_{2B}-6 clone (401% and 334%, respectively). Another potent α_2-adrenoceptor agonist, UK14,304, was also tested with the α_{2B}-6 clone. UK14,304 stimulated cAMP production by 382% in the α_{2B}-6 clone.

Treatment of the cells with 5 μM forskolin for 10 min resulted in 20- to 50-fold increases in the rate of cAMP production (data not shown). In cells expressing the α_{2C}-subtype the ligands inhibited cAMP production in all clones (Table 4). The maximal inhibitory effect in the α_{2C}-L2 clone was 39% for (−)-noradrenaline and 27% for dexmedetomidine, but clonidine and levomedetomidine had no significant effects. In the α_{2C}-C1, α_{2C}-L3 and α_{2C}-R1-6 clones the maximal inhibitory effects of (−)-noradrenaline, dexmedetomidine and clonidine were 90%, and even levomedetomidine inhibited cAMP production by 50–60%. In the α_{2B}-3 clone, no significant change in the forskolin-stimulated cAMP production was induced by (−)-noradrenaline, but a small inhibitory effect was seen with dexmedetomidine (12%) (Table 4). In the α_{2B}-6 clone, the forskolin-stimulated cAMP production was inhibited by (−)-noradrenaline (47%), UK14,304 (52%) and dexmedetomidine (24%).

The effects of 100 μM UK14,304 and dexmedetomidine in the α_{2B}-6 clone were further investigated in the presence of different concentrations of forskolin. Fig. 2 shows the forskolin-induced dose-dependent increase in the conversion of [^{3}H]ATP to [^{3}H]cAMP. The effects of 100 μM UK14,304 (Fig. 2a) or 100 μM dexmedetomidine (Fig. 2b) on cAMP production were dependent on the concentration of forskolin used. In the absence of forskolin, UK14,304 and dexmedetomidine stimulated cAMP production (by 280% and 200%, respectively), but the stimulatory effect declined with increasing concentrations of forskolin, and at high concentrations of forskolin (1–5 μM) UK14,304 and dexmedetomidine inhibited cAMP

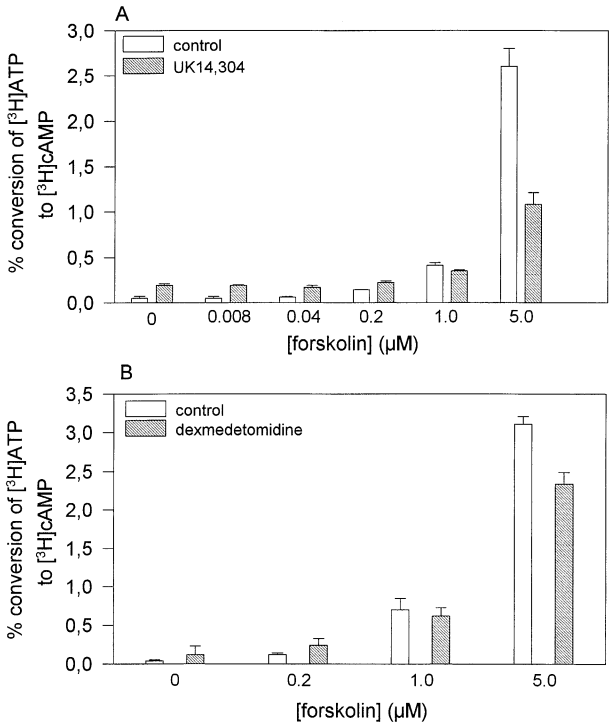

Fig. 2. The effect of different concentrations of forskolin alone and in the presence of UK14,304 (100 μM) (A) and dexmedetomidine (100 μM) (B) on the % conversion of [^{3}H]ATP to [^{3}H]cAMP in intact CHO-α_{2B}-6 cells. Results are means ± S.E. from 2 experiments performed in triplicate.

Table 4
α_{2B}- and α_{2C}-adrenoceptor-mediated effects on cAMP production in intact CHO cells

CHO clone	E_{max}					
	α_{2B}-3	α_{2B}-6	α_{2C}-C1	α_{2C}-L2	α_{2C}-L3	α_{2C}-R1-6
Ligand − forskolin:						
Noradrenaline	31.7 ± 1.7	401 ± 92	nd	nd	nd	nd
Dexmedetomidine	41.7 ± 8.3	334 ± 139	nd	nd	nd	nd
UK14,304	nd	382 ± 90.5	nd	nd	nd	nd
Ligand + forskolin:						
Noradrenaline	8.2 ± 8.8(ns)	−47.2 ± 15.7	−92.7 ± 2.4	−38.6 ± 11.5	−94.0 ± 2.8	−94.4 ± 2.4
Dexmedetomidine	12.2 ± 8.7	−23.7 ± 3.1	−89.2 ± 2.2	−26.9 ± 1.8	−93.9 ± 3.2	−92.5 ± 4.3
Levomedetomidine	nd	nd	−46.3 ± 30.8	−3.85 ± 3.9	−53.0 ± 7.6	−61.9 ± 4.9
Clonidine	nd	nd	−90.9 ± 6.1	−15.5 ± 7.8	−92.5 ± 5.3	−89.3 ± 4.6
UK14,304	nd	−51.7 ± 11.8	nd	nd	nd	nd

The % conversion of [^{3}H]ATP to [^{3}H]cAMP was measured in intact CHO cells expressing α_{2B}- and α_{2C}-adrenoceptors. The maximal responses (E_{max}) are expressed as % change from enzyme activity in the absence of agonists. Positive values indicate % stimulation and negative values indicate % inhibition. + or − forskolin indicates presence or absence of 5 μM forskolin. The values are ± S.E. from 2–4 experiments performed in triplicate. The statistical significance as in Table 2. nd, not determined.

production (by 59% and 25%, respectively, in the presence of 5 μM forskolin).

4. Discussion

Regulation of adenylyl cyclase activity and cellular cAMP concentrations plays an important role in the control of many cellular functions, including gene expression and integration of hormonal stimulation (Gilman, 1990). In this study, the α_2-adrenoceptor-mediated regulation of adenylyl cyclase activity was investigated in transfected CHO cells. The α_2-adrenoceptor subtypes, α_{2A}, α_{2B} and α_{2C}, were expressed at different densities and could be distinguished by competition binding assays with the subtype-selective ligand oxymetazoline. The different α_2-adrenoceptor subtypes showed the same rank order of apparent binding affinity for the ligands tested, dexmedetomidine > clonidine = levomedetomidine > (−)-noradrenaline. The same rank order for these ligands has been presented in previous studies (Jansson et al., 1994a, 1995). In this study only small GTP-induced rightward shifts of the high-affinity components of competition binding curves could be seen, and no clear differences could be observed between the full agonist (−)-noradrenaline and the partial agonist clonidine. This indicates that binding assays performed in the presence and absence of Gpp(NH)p would not, in these cells, be predictive of agonist efficacy.

Another way to predict the efficacy of a ligand should be to calculate the ratio of the binding constants for binding to the low- and high-affinity states of the receptor (K_{iL}/K_{iH}) (Kenakin, 1993). Calculation of this ratio for the ligands tested identified clear differences. (−)-Noradrenaline had the highest ratio for all receptor subtypes, dexmedetomidine and clonidine had intermediate ratios and levomedetomidine had the lowest ratio for all subtypes. Only small differences could be seen in the efficacy of the different agonists for inhibiting adenylyl cyclase activity at low densities of the α_{2A}- and α_{2B}-adrenoceptors in isolated cell membranes ((−)-noradrenaline > dexmedetomidine > clonidine > levomedetomidine). In membranes containing α_{2C}-adrenoceptors, clearer differences between the ligands could be seen in inhibition of forskolin-stimulated adenylyl cyclase activity (−)-noradrenaline = dexmedetomidine > clonidine > levoedetomidine), irrespective of receptor density. Clear differences in agonist efficacy could also be seen in coupling to stimulation of adenylyl cyclase in all clones expressing α_{2B}-adrenoceptors ((−)-noradrenaline > dexmedetomidine > clonidine > levomedetomidine). Therefore, it seems that the K_{iL}/K_{iH}-ratio can be used to predict agonist efficacy. Such a simple assay could be very useful in the screening of new agonist ligands.

The coupling of the receptor subtypes to adenylyl cyclase was investigated in both membrane preparations and in intact cells. The agonist efficacies were clearly lower in isolated membranes than in intact cells. One reason could be that the sensitivity of signal transduction is partially destroyed during the preparation of membranes. Because of the relatively low efficacies of the different agonists in isolated membranes, it was somewhat difficult to classify the ligands as full or partial agonists. In intact cells, this should have been easier since clearly higher and more variable efficacies were obtained. In the α_{2C}-L2 clone, which expresses a low level of α_{2C}-adrenoceptors, clonidine and levomedetomidine inhibited forskolin-stimulated cAMP production with lower efficacy than (−)-noradrenaline and dexmedetomidine. This result is in agreement with a prediction based on the K_{iL}/K_{iH}-ratio, and is also in agreement with previous results demonstrating that clonidine is a partial agonist for all α_2-adrenoceptor subtypes (Jansson et al., 1994a,b, 1995). In contrast, in the clones expressing high levels of α_{2C}-adrenoceptors (0.6–2 pmol/mg) clonidine acted as a full agonist, by inhibiting cAMP accumulation with an efficacy equal to that of (−)-noradrenaline and dexmedetomidine. Therefore, it seems important that the receptor density is not exceedingly high when attempting to classify α_2-ligands as full or partial agonists. It has been demonstrated that receptor reserve can mask partial agonist activity (Adham et al., 1993), which can result in a misleading classification of ligands.

Interestingly, dexmedetomidine did not act as a complete full agonist for either stimulation of adenylyl cyclase activity or inhibition or stimulation of cAMP production in cells expressing α_{2B}-adrenoceptors. In previous studies by Jansson et al. (1994a, 1995) with transfected cell lines, dexmedetomidine acted as a full agonist at all three receptor subtypes. This classification of dexmedetomidine could be misleading as a result of the high level of receptor expression, because in a study with intact HEL cells, endogenously expressing a low density of α_{2A}-adrenoceptors (70–130 fmol/mg) (McKernan et al., 1987; Musgrave and Seifert, 1995), a clear rank order of efficacy for inhibition of forskolin-stimulated cAMP production could be seen, i.e., (−)-noradrenaline > dexmedetomidine > clonidine (Jansson et al., unpublished data). This partial agonist effect by dexmedetomidine has also been demonstrated in Chinese hamster lung fibroblasts expressing relatively low levels of α_{2A}- (470 fmol/mg) and α_{2C}- (240 fmol/mg) adrenoceptors (Jansson et al., 1994b). Levomedetomidine, which is known to bind to α_2-adrenoceptors and to be inactive in various in vivo models and to be a very weak agonist in various in vitro models (Savola and Virtanen, 1991; Jansson et al., 1994a,b, 1995), was in this study almost inactive in the membrane assay, but could inhibit forskolin-stimulated cAMP production with an efficacy as high as 50–60% in intact cells expressing 0.7–2 pmol/mg α_{2C}-adrenoceptors. The receptor densities were quite high, which undoubtedly partially masked the weak activity of levomedetomidine, but the results still demonstrate that levomedetomidine can act as a partial

agonist in some systems. This is of interest, because in another study performed with HEL cells it was demonstrated that levomedetomidine acted as an inverse agonist, by reducing basal receptor activity (Jansson et al., unpublished data). It has been suggested that receptor ligands that in some systems act as inverse agonists, and in other systems as agonists, should be named protean agonists (Kenakin, 1996). Together with the study performed with HEL cells, our study supports the view that levomedetomidine is a protean agonist.

Our results indicate that the α_{2B}-subtype clearly differs from the other two α_2-adrenoceptor subtypes in its coupling to adenylyl cyclase. In contrast with the α_{2A}- and α_{2C}-adrenoceptors, activation of α_{2B}-adrenoceptors resulted in stimulation of both adenylyl cyclase activity and cAMP production in the absence of forskolin. This result is of interest because in other studies demonstrating that the α_{2B}-subtype can stimulate cAMP production, this effect was only seen in the presence of forskolin (Duzic and Lanier, 1992; Pepperl and Regan, 1993; Jansson et al., 1994a, 1995). In our study all subtypes were coupled to inhibition of adenylyl cyclase and cAMP production in the presence of forskolin, which is in agreement with the results of another study performed with CHO cells (Eason et al., 1992). However, we saw no evidence of α_{2A}- and α_{2C}-adrenoceptors mediating stimulation of adenylyl cyclase activity after pertussis toxin treatment, which is in clear contrast with the findings of Eason et al. (1992). In our study, only the α_{2B}-subtype mediated stimulation of adenylyl cyclase after pertussis toxin treatment. In the study by Eason et al. the effects of agonists were unfortunately not tested in the absence of forskolin.

Our results also demonstrate that the coupling of the α_{2B}-subtype to regulation of adenylyl cyclase depends on the level of activity of adenylyl cyclase. At low levels of activity, activation of the α_{2B}-subtype resulted in stimulation of adenylyl cyclase, but when adenylyl cyclase was already activated by forskolin the activation of α_{2B}-adrenoceptors resulted in inhibition of enzyme activity. After pertussis toxin treatment the inhibitory transduction pathway was blocked, and independently of the adenylyl cyclase activity only stimulatory effects could be detected.

In a study by Jansson et al. (1995) it was demonstrated that the stimulatory effect transduced by the α_{2B}-subtype in intact Sf9 cells was not affected when several possible transduction mechanisms were blocked (G_i was blocked with pertussis toxin, intracellular Ca^{2+} was chelated with BAPTA/AM, protein kinase C was blocked with staurosporine, phospholipase A_2 was blocked with quinacrine and activation of Na^+/H^+ exchange was inhibited by amiloride). The α_{2B}-mediated stimulation of cAMP production was, however, blocked by cholera toxin, indicating mediation of the effect through G_s proteins. The present study also supports a direct coupling of α_{2B}-adrenoceptors to G_s, because the stimulatory effect was seen also in isolated membrane preparations and was not sensitive to pertussis toxin, excluding the possibility of stimulation of type II or type IV adenylyl cyclases by the $\beta\gamma$-subunits released from the activated G_i protein. The low inhibitory efficacy of the α_{2B}-subtype in intact cells can therefore be explained by simultaneous coupling to G_s and G_i. Coupling to stimulation of adenylyl cyclase through activation of phospholipase A_2, which has been demonstrated at high α_2-agonist concentrations in intact CHO cells expressing the α_{2A}-subtype (Fraser et al., 1989), can be excluded because all experiments with intact cells were performed in the presence of quinacrine.

In conclusion, the present results suggest that the α_{2B}-adrenoceptor subtype is coupled to both G_i and G_s proteins. The signal transduction pathway to which the receptor is coupled is not dependent on receptor density, but its effect on adenylyl cyclase regulation is dependent on the current activity of adenylyl cyclase. The results also suggest that the α_{2A}- and α_{2C}-subtypes preferentially couple to G_i and mediate only inhibition of adenylyl cyclase activity in transfected CHO cells.

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