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α_{2B}-Adrenoceptor levels govern agonist and inverse agonist responses in PC12 cells

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Abstract

Receptor density is an important determinant of cellular effector responses to receptor activation. We analysed cytosolic Ca^{2+} responses to α_2-adrenergic agents in PC12 cells expressing human α_{2B}-adrenergic receptors (AR) at two densities (3.8 and 1.3 pmol/mg protein). The efficacy (E_{max}) of agonists was greater in cells with higher receptor expression; while the potency (EC_{50}) of norepinephrine and oxymetazoline was independent of α_{2B}-AR levels. Several classical α_2-AR antagonists behaved as either partial or inverse agonists in a receptor density-dependent fashion. No apparent structural similarities were found among the inverse agonists, precluding simple predictions of inverse agonist activity. Transfected PC12 cells expressing α_{2B}-AR at relatively high density would be a useful approach to screen inverse agonists for this class of receptors. Our results further indicate that receptor density significantly influences the properties of ligands, not only of partial agonists as predicted by classical receptor theory, but also of antagonists and full agonists.

Keywords: α_{2B}-Adrenoceptor; Expression level; Inverse agonism; Ca^{2+} signalling; PC12 cells

Three human α_2-adrenoceptor (α_2-AR) subtypes (α_{2A}, α_{2B}, and α_{2C}) have been identified; they are differently distributed in nervous and non-nervous tissues and mediate multiple physiological and pharmacological effects, such as sedation, antinociception, bradycardia, and regulation of blood pressure [1,2]. Activation of the α_{2B}-AR subtype has been reported to increase the release of Ca^{2+} from intracellular stores and to inhibit cAMP production via $G_{i/o}$-type G proteins, but also to increase cAMP production via a G_s-dependent mechanism [3,4].

Efforts have been made to quantitatively describe the relationship between receptor density and functional responses to agonists. The classical theory of drug–receptor interaction predicts that an increase in receptor density will first lead to an increase in the maximal efficacy of an agonist. Further increase in receptor density will, however, no longer raise the maximal response, due to restrictions imposed by limitations in the capacity of downstream elements in the signalling cascade involved, but will increase the apparent potency of the agonist. The maximal response is now obtained with a lower concentration of agonist. This phenomenon is referred to as 'receptor reserve' [5–7]. However, the relationships between receptor density and agonist efficacy and potency have not always exactly followed the predicted patterns in transfected cell models expressing various types of adrenoceptors. When hamster and human β_2-AR were expressed at widely varying densities in Ltk^- fibroblast cells, and stimulation of adenylyl cyclase activity was used as the output measure, good agreement was found between predictions based on receptor density and the actual observed E_{max} and EC_{50} values of epinephrine [8]. On the other hand, when studied over a more narrow range of expression levels, increasing the expression of β_1-AR caused higher apparent potency of agonists in stimulation of cAMP production, without changes in E_{max} values, in rat C6 glioma cells [9] and in GH3 pituitary cells [10]. Similar observations were also reported for β_2-AR in GH3 cells

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[10]. On the contrary, a linear correlation was plotted between α_{1B}-AR density and E_{max} rather than EC_{50} of norepinephrine (NE) in stimulation of inositol phosphate formation in DDT_1 MF-2 cells [11]. Similar results were obtained with intracellular Ca^{2+} assays for α_{1B}-AR in CHO cells [12]. In our previous study, high-level expression of all three α_2-AR subtypes increased E_{max} and reduced EC_{50} of NE in inhibition of adenylyl cyclase activity, but high-level expression of α_{2B}-AR only increased E_{max} of NE in stimulation of adenylyl cyclase activity in CHO cells with no effect on potency [4]. The apparent discrepancies between these studies may be explained in the context of classical drug–receptor action if one takes into account that an effect on potency only becomes apparent after a maximum has been reached in the evoked response.

Inverse agonists have recently generated interest in clinical therapeutics and in drug design and discovery. How receptor density influences the pharmacological properties of inverse agonists is still an open question. The distinction between agonists, antagonists, and inverse agonists appears to depend on receptor expression levels. For example, the dopamine D_4 receptor antagonists, U-101958 and L-745,870, started to mimic dopamine at inhibiting forskolin-stimulated cAMP accumulation in CHO cells when receptor density was increased [13]. At β_2-AR, all tested antagonists were found to inhibit agonist-independent cAMP accumulation (order of inverse efficacy: timolol $\geqslant$ propranolol > alprenolol > pindolol > labetalol > dichloroisoproterenol) when β_2-AR expression was increased to 40 pmol/mg protein [14].

It is expected that high levels of receptor expression provide high basal signalling activity, making inverse agonism more easily detectable. However, the cases of detectable inverse agonism in cell models with overexpression of wild-type adrenoceptors are quite rare [14,15]. So far, neither theoretical considerations nor practical assay methods have been able to elucidate the relationships between variable receptor expression levels and the functional properties of antagonists—the structural determinants of neutral antagonism vs. inverse agonist activity are generally unknown. It was therefore considered to be important to develop a convenient model system to screen α_2-adrenergic ligands for detection of inverse agonist activity.

In the present study, we employed PC12 cells stably transfected to express human α_{2B}-AR at two distinct expression levels (3.8 and 1.3 pmol/mg protein) to test how receptor density affects the pharmacological profiles of several representative full and partial α_2-agonists as well as antagonists by measuring time-dependent intracellular Ca^{2+} responses. PC12 cells were originally derived from a rat pheochromocytoma and are widely used as a model system in receptor pharmacology.

Materials and methods

Materials. Materials were purchased from the following sources: Dulbecco's modified Eagle's medium (DMEM), horse serum (HS), and fetal bovine serum (FBS) from Gibco-BRL (Gaithersburg, MD); Vitrogen 100 from Collagen (Fremont, CA); fura 2/acetoxymethylester (fura 2/AM) from Molecular Probes Europe BV (Leiden, The Netherlands); epinephrine, ARC 239, chlorpromazine, clonidine, digitonin, EGTA, NE, oxymetazoline HCl, pertussis toxin (PTX), phentolamine, rauwolscine, and 2-{[3-hydroxyl-1,1-bis(hydroxymethyl) ethyl]amino} ethane sulphonate (TES) from Sigma Chemical (St. Louis, MO); and RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline) from Research Biochemicals International (Natick, MA). (2S,12bS)1′,3′-dimethylspiro (1,3,4,5′,6,6′,7,12b-octa hydro-2H-benzo[b]furo[2,3-a] quinazoline)-2,4′-pyrimidin-2′-one (MK912) was a gift from Merck, Sharp and Dohme Research (West Point, PA), and atipamezole, dexmedetomidine, and levomedetomidine were gifts from Orion Corporation Orion Pharma (Turku, Finland). Other chemicals were obtained from commercial suppliers.

Cell lines and culture. PC12 cells were transfected with a pMAMneo (Clontech, Palo Alto, CA)-based expression vector containing the cDNA for the human α_{2B}-AR [16] and cultured as previously described [17]. PC12α_{2B}-9 (expressing human α_{2B}-AR at 3.8 pmol/mg total cellular protein) and PC12α_{2B}-8 (1.3 pmol/mg protein) were used for $[Ca^{2+}]_i$ measurements. For some experiments, cells were cultured in serum-free medium supplemented with PTX 250 ng/ml for 12 h.

Measurement of intracellular Ca^{2+} concentration. Alterations in $[Ca^{2+}]_i$ were measured with the fluorescent indicator fura 2/AM. Cells were gently collected with a rubber scraper. The culture medium was replaced with experimental medium (EM) containing (mM): NaCl, 137; KCl, 5; $NaHCO_3$, 4.2; $MgCl_2$, 1.2; glucose, 10; $CaCl_2$, 1; KH_2PO_4, 0.44; and TES, 20, pH 7.4. Fura 2/AM (4 μg/ml) was loaded into the cells for 30 min at 37 °C. Then, the cells were washed once, suspended in EM, and kept at room temperature for use. Aliquots of cell suspension were transferred into a stirred cuvette at 37 °C. Fluorescence was continuously monitored with a fluorescence spectrophotometer (Hitachi F-2000) with excitation and emission wavelengths of 340 and 510 nm, respectively. After applying ligands and recording of responses, fluorescence records were calibrated by addition of digitonin (60 μg/ml) and EGTA (10 mM) to obtain maximal and minimal values of fluorescence, respectively. $[Ca^{2+}]_i$ was calculated according to the equation:

$$[Ca^{2+}]_i = (F - F_{min})/(F_{max} - F) * 224\,nM$$

with extracellular fura-2 fluorescence subtracted from all F values [18].

Data analysis. EC_{50} and E_{max} were determined by non-linear regression analysis using the software Prism III (GraphPad, San Diego, CA). Data were fitted as sigmoidal concentration–response curves. The statistical significance of the observed differences between PC12α_{2B}-9 and PC12α_{2B}-8 cells was determined with unpaired t tests; p-values of less than 0.05 were considered as significant.

Results

Agonist-induced Ca^{2+} responses

The basal cytosolic Ca^{2+} concentration was approximately 100 nM in non-transfected PC12 cells and in stably transfected PC12 cells expressing human α_{2B}-AR. The maximal Ca^{2+} efficacies (E_{max}) to all studied agonists were somewhat greater in PC12α_{2B}-9 than in PC12α_{2B}-8 cells (Table 1; Fig. 1). The E_{max} of full α_2-AR agonists (NE, epinephrine, and dexmedetomidine)

Table 1
Comparison of the potencies and efficacies of α_2-AR agonists in PC12 cells transfected to express α_{2B}-AR at densities of 3.8 and 1.3 pmol/mg protein

Agonist	PC12α_{2B}-9 cells (3.8 pmol/mg)			PC12α_{2B}-8 cells (1.3 pmol/mg)		
	EC_{50}	E_{max}	Relative efficacy (% of response to NE)	EC_{50}	E_{max}	Relative efficacy (% of response to NE)
(−)-Norepinephrine	27 ± 4	$239 \pm 17^{**}$	100	29 ± 11	164 ± 8	100
(−)-Epinephrine	101 ± 18	$241 \pm 20^{**}$	100	140 ± 15	146 ± 8	89
Dexmedetomidine	$0.72 \pm 0.06^{***}$	$329 \pm 14^{***}$	138	2.0 ± 0.07	163 ± 13	99
Oxymetazoline	44 ± 7	182 ± 25	76	47 ± 17	131 ± 13	80
Clonidine	19 ± 7	$64 \pm 4^{*}$	26	39 ± 6	49 ± 2	30
Levomedetomidine	$163 \pm 57^{*}$	$75 \pm 6^{**}$	31	291 ± 123	42 ± 5	26

The values are means ± SEM (nM) of four independent experiments performed at least in duplicate.
$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to PC12α_{2B}-8 cells.

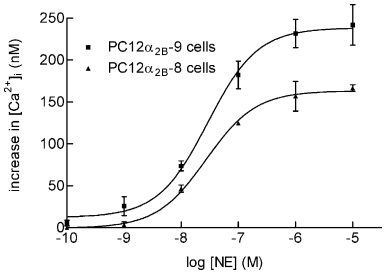

Fig. 1. Concentration–response curves for $[Ca^{2+}]_i$ increases induced by NE in PC12α_{2B}-9 and PC12α_{2B}-8 cells. Data are means ± SEM of four independent experiments performed in duplicate.

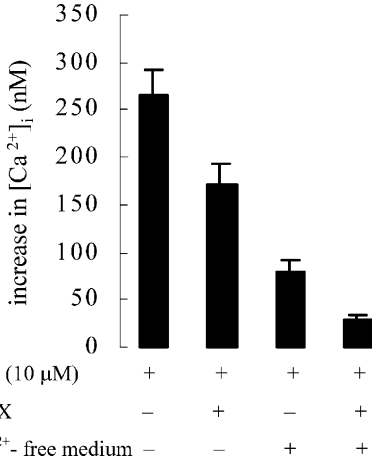

Fig. 2. Effects of PTX and Ca^{2+}-free medium on NE-induced Ca^{2+} responses in PC12α_{2B}-9 cells. Data are means ± SEM of at least two independent experiments performed in triplicate or quadruplicate.

and of partial agonists (oxymetazoline, clonidine, and levomedetomidine) were increased approximately 1.5-fold in PC12α_{2B}-9 compared with PC12α_{2B}-8 cells, but the relative efficacies of the agonists (compared to NE) were not markedly influenced by the receptor expression level (Table 1). The potencies (EC_{50}) of NE and oxymetazoline were not affected by the expression levels of α_{2B}-AR (Table 1 and Fig. 1). The potencies of epinephrine and clonidine tended to be somewhat greater when receptor expression was high; those of dexmedetomidine and levomedetomidine were significantly increased (by 3- and 1.5-fold, respectively) in PC12α_{2B}-9 compared to PC12α_{2B}-8 cells (Table 1). As a negative control, parental PC12 cells (non-transfected) were employed to examine whether the tested agonists have non-α_{2B}-AR mediated effects on cytosolic Ca^{2+} release. No $[Ca^{2+}]_i$ elevations were detected after addition of NE, dexmedetomidine, or oxymetazoline (data not shown).

The involvement of $G_{i/o}$-type G-proteins in the effects of α_2-AR agonists on Ca^{2+} signalling in PC12 cells was investigated by using PTX, a potent and irreversible inhibitor of $G_{i/o}$ function. Pretreatment with PTX blocked only about 30% of the maximal Ca^{2+} response to NE (10 μM) in both PC12α_{2B}-9 (Fig. 2) and PC12α_{2B}-8 cells (data not shown). These results suggested that α_{2B}-AR-mediated Ca^{2+} release is only partly dependent on the $G_{i/o}$ pathway and that the involvement of $G_{i/o}$ is proportional to receptor density. When Ca^{2+}-free medium was used, 70% of the maximal agonist-dependent Ca^{2+} mobilisation was abolished. In conditions of combined PTX pretreatment and Ca^{2+}-free assay buffer, a small Ca^{2+} response was still detectable after agonist administration (11% of the maximal response; Fig. 2).

Inverse agonist effects are more evident at high α_{2B}-AR expression levels

The influence of seven α_2-AR antagonists on cytosolic Ca^{2+} levels in PC12α_{2B}-9 and PC12α_{2B}-8 cells was examined to investigate whether transfected PC12 cells expressing human α_{2B}-AR at high density would provide a test system for the detection of inverse agonist activity at α_{2B}-AR. The results displayed great diversity.

Chlorpromazine, rauwolscine, and RX821002 displayed negative efficacy as demonstrated by their ability to decrease $[Ca^{2+}]_i$ in α_{2B}-AR-expressing PC12 cells (Fig. 3). This apparent inverse agonist efficacy of these three compounds was greater in PC12α_{2B}-9 than in PC12α_{2B}-8 cells (Table 2). The neutral antagonist MK912

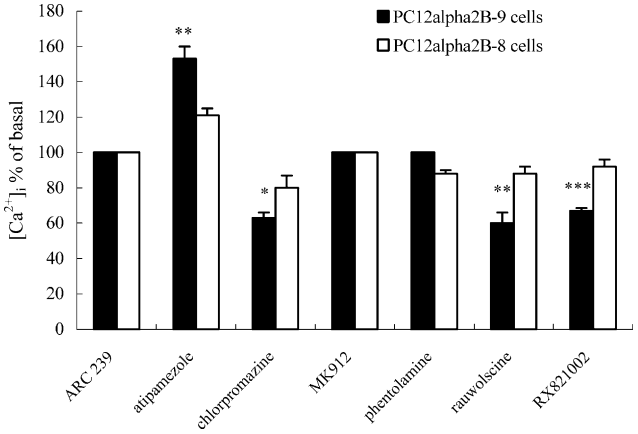

Fig. 3. Antagonist-induced changes in $[Ca^{2+}]_i$ compared to basal $[Ca^{2+}]_i$ in PC12α_{2B}-9 (closed columns) and PC12α_{2B}-8 (open columns) cells. Fluorescence intensity was recorded 3 min after addition of 300 nM of each antagonist. Data are means ± SEM of at least three independent experiments performed in duplicate. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$ for comparison between PC12α_{2B}-9 and PC12α_{2B}-8 cells.

both depressed dexmedetomidine-evoked Ca^{2+} mobilisation (Fig. 4) and completely blocked the negative efficacy of chlorpromazine, rauwolscine, and RX821002 (data not shown). Pretreatment with PTX was unable to prevent the negative Ca^{2+} response to RX821002 (Fig. 5). Non-transfected PC12 cells were again employed to examine whether chlorpromazine, rauwolscine, and RX821002 could influence cytosolic Ca^{2+} concentrations in PC12 cells in a manner independent of α_{2B}-AR expression. No alterations in basal $[Ca^{2+}]_i$ were observed after administration of these compounds, confirming that also the inhibitory effects of these drugs are mediated by

Table 2
α_2-Antagonist-induced decreases (%) of cytosolic Ca^{2+} concentrations in PC12α_{2B}-9 and PC12α_{2B}-8 cells

Antagonist (nM)	PC12α_{2B}-9	PC12α_{2B}-8
Chlorpromazine		
5	13 ± 6	6 ± 6
30	21 ± 2**	4 ± 3
100	28 ± 3**	5 ± 5
300	39 ± 3*	21 ± 7
Rauwolscine		
5	7 ± 3*	0 ± 0
30	24 ± 3*	8 ± 5
100	35 ± 4*	16 ± 7
300	40 ± 6**	18 ± 5
RX821002		
5	24 ± 3**	6 ± 4
30	30 ± 3***	7 ± 1
100	27 ± 6	12 ± 3
300	29 ± 2***	9 ± 3

The values are means ± SEM of at least four independent experiments performed in duplicate.

$^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to PC12α_{2B}-8 cells.

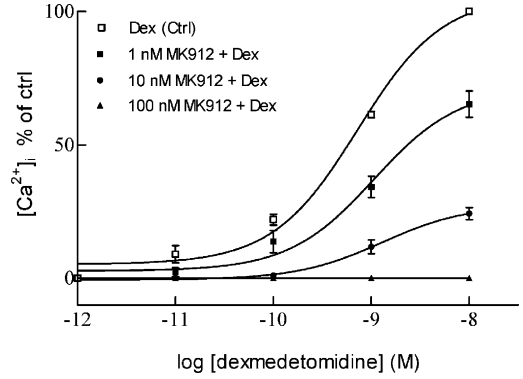

Fig. 4. Effect of the neutral antagonist MK912 on dexmedetomidine-induced Ca^{2+} responses in PC12α_{2B}-9 cells. MK912 was added 3 min before dexmedetomidine challenge. Data are means ± SEM of four separate experiments performed in duplicate.

α_{2B}-AR, rather than by other unknown effects on Ca^{2+} signalling. We further investigated how an inverse agonist influences the agonist-induced Ca^{2+} release. Varying concentrations of RX821002 shifted the concentration–response curve of the full agonist NE to the right in both PC12α_{2B}-9 and PC12α_{2B}-8 cells, but the depression in the maximal response to NE was larger (approximately 50%) in PC12α_{2B}-9 cells (Fig. 6A) than in PC12α_{2B}-8 cells (Fig. 6B).

In contrast to the three inverse agonists identified above, which suppressed the basal Ca^{2+} levels, atipamezole, one of the tested antagonists, appeared to increase cytosolic Ca^{2+} levels in a receptor density- and concentration-dependent manner (Fig. 7). In PC12α_{2B}-9 cells, the maximal $[Ca^{2+}]_i$ after atipamezole was about 40 nM, while in PC12α_{2B}-8 cells, atipamezole evoked only weak-agonist-like responses (Fig. 7). The stimulatory effect of atipamezole on cytosolic Ca^{2+} release was

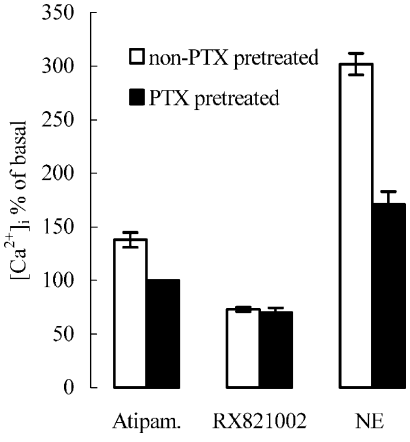

Fig. 5. Effect of PTX on Ca^{2+} responses to atipamezole (300 nM), RX821002 (300 nM), and NE (10 μM) in PC12α_{2B}-9 cells. Data are means ± SEM of at least two independent experiments performed in duplicate or triplicate for non-PTX pretreated cells (open columns) and in quadruplicate for PTX-pretreated cells (closed columns).

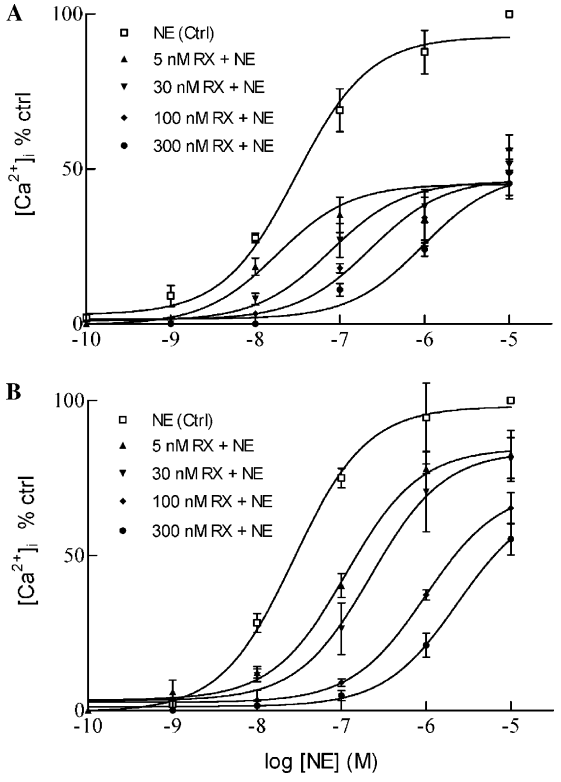

Fig. 6. Effect of RX821002 on NE-induced Ca^{2+} responses in PC12α_{2B}-9 (A) and PC12α_{2B}-8 cells (B). RX821002 was added 3 min before application of NE. Data are means ± SEM of four separate experiments performed in duplicate.

abolished by pretreatment with PTX (Fig. 5) and also by co-incubation with the neutral antagonist MK912 (100 nM) (data not shown). No increases in $[Ca^{2+}]_i$ to atipamezole (300 nM) were seen in non-transfected PC12 cells.

The remaining compounds, ARC 239, an α_{2B}-selective antagonist, MK912, and phentolamine, altered neither positively nor negatively cytosolic Ca^{2+} levels in α_{2B}-AR-expressing PC12 cells, and emerged as neutral antagonists (Fig. 3).

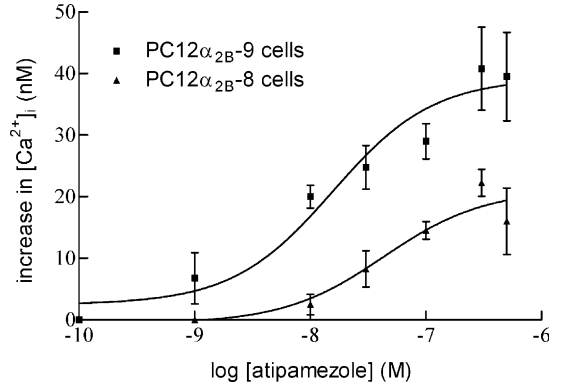

Fig. 7. Concentration–response curves for $[Ca^{2+}]_i$ increases induced by atipamezole in PC12α_{2B}-9 and PC12α_{2B}-8 cells. Data are means ± SEM of four independent experiments performed in duplicate.

Discussion

During the past decades, great efforts have been made to understand the consequences of varied receptor densities on the maximal efficacy (E_{max}) and potency (EC_{50}) of agonists for stimulating cellular response systems. Although these relationships may be accurately predicted in simple systems, experimental results have not always followed the predictions for various reasons. Furthermore, the effects of receptor density on the functional properties of antagonists are generally uncertain. In this study, we demonstrated that structurally different agonists produced different results when the influence of varied α_{2B}-AR expression levels was assessed on the potency of α_2-AR agonists. The EC_{50} values of NE and oxymetazoline remained constant in both cell lines; whereas the potency of dexmedetomidine was significantly increased along with increased α_{2B}-AR expression. We also demonstrated that high-level expression of α_{2B}-AR provided a useful approach to distinguish between inverse agonists and neutral antagonists, and also between very weak agonists and neutral antagonists. Furthermore, the results showed that the density of recombinant α_{2B}-AR influenced the apparent pharmacological profiles of the tested α_2-AR antagonists in very different fashions, the responses ranging from partial agonism (atipamezole) to neutral antagonism (ARC 239, MK912, and phentolamine), and finally to inverse agonism (chlorpromazine, rauwolscine, and RX821002) in cells with a high receptor expression level.

The apparent potency of NE to induce Ca^{2+} responses in PC12 cells was not influenced by α_{2B}-AR density, but the maximal responses to the catecholamines NE and epinephrine were proportional to the receptor expression level (Table 1). Similar findings for NE have been detected earlier with other ARs. Despite a 70-fold increase in the density of α_{1B}-AR in CHO cells, NE-induced Ca^{2+} responses showed similar EC_{50} values while the maximal response was increased about 6-fold [12]. Increasing α_{1B}-AR expression in DDT_1 cells caused a linear increase in the maximal response to NE-stimulated inositol phosphate formation, but the EC_{50} values remained essentially constant [11]. Our previous work showed that the potency of NE did not change when stimulatory adenylyl cyclase activity was studied in CHO cells with varying densities of α_{2B}-AR expression. However, both increased maximal efficacies and potencies of NE were observed in inhibitory adenylyl cyclase regulation with all three human α_2-AR subtypes in a receptor density-dependent manner [4]. It is not clear why our present results for dexmedetomidine were different from the other agonists in terms of potency. One possibility is that the active receptor conformation stabilized by dexmedetomidine is sufficiently different from those favoured by the other agonists to induce different G-protein coupling, resulting in different

stoichiometries in the resulting receptor–G-protein–effector cascades.

Our results are in general consistent with predictions based on classical theory of receptor action [5,7] and with the results of our previous study on recombinant human α_2-AR subtypes expressed in CHO cells [4]. In terms of classical receptor theory, the first determinant of the response to an agonist stimulus is receptor density. The higher the receptor number, the larger the capacity of the drug–receptor–transducer complex. Once maximal efficacy is reached, a further increase in receptor number will enhance the apparent potency of the agonist. In this study, all tested agonists displayed approximately 1.5-fold higher maximal responses in PC12α_{2B}-9 than in PC12α_{2B}-8 cells, demonstrating that an amplified signalling input occurs in the drug–receptor–transducer complex. Significantly increased potency was observed only for dexmedetomidine and levomedetomidine, indicating that a threshold was reached in PC12α_{2B}-9 cells, where downstream components of the signalling cascade started to limit the evoked responses. Receptor reserve was thus apparent for these agonists. Moreover, an increased receptor reserve should increase the apparent intrinsic activity of partial agonists [5,19]. Partial agonists are defined as drugs that produce only a submaximal response at maximal occupation of receptors. Several previous studies have demonstrated that partial agonists start to behave like full agonists when receptor expression is sufficiently increased [4,20]. In the present study, the intrinsic activities of the weak partial agonists, clonidine and levomedetomidine, did not approach those of the full agonists when receptor expression was increased. Only approximately 30% of maximal responses were reached in both cell lines. Thus, it appears that the receptor density in PC12α_{2B}-9 cells was not high enough to cause masking of partial agonist activity, although it was sufficiently high to reveal the partial agonist characteristics of atipamezole, which has behaved as a neutral antagonist in other pharmacological assay systems [21,22]. The weak partial agonist properties of atipamezole are apparently not detectable with all experimental systems. The high α_{2B}-AR density in our PC12α_{2B}-9 cells and the availability of a suitable amplification system allowed detectable receptor-mediated signalling with similar mechanisms as for other weak partial agonists. Similar unmasking of agonist properties by a high receptor density has previously been reported for the dopamine D_4 receptor [13].

Dexmedetomidine emerged as a super-agonist in PC12 cells expressing α_{2B}-AR at high density. It evoked equal maximal responses as the natural agonist NE in PC12α_{2B}-8 cells, but consistently induced about 40% larger maximal responses than the catecholamines in PC12α_{2B}-9 cells. Its potency was also increased about 3-fold in PC12α_{2B}-9 compared to PC12α_{2B}-8 cells.

The apparent inverse agonist characteristics of chlorpromazine, rauwolscine, and RX821002 were dependent upon the density of α_{2B}-AR in stably transfected PC12 cells. Tian and his colleagues [15] observed that rauwolscine, yohimbine (an isomer of rauwolscine), idazoxan (from which RX821002 is derived), and phentolamine reduced basal GTPγS binding in PC12 cell membranes expressing the rat α_{2A}-AR subtype. The magnitude of the inverse agonist effect of rauwolscine was linearly related to the α_{2A}-AR density over a range of 0.37–3.6 pmol/mg membrane protein in this direct assay of inverse agonism. Based on the present results and those of Tian et al. [15], we would like to suggest that high levels of receptor expression provide an improved possibility to detect inverse agonist activity at receptors that normally exhibit only very low levels of constitutive activity. The structural determinants of negative efficacy have not been elucidated. Obviously, some antagonist ligands are more efficacious than others in stabilising an inactive receptor conformation and in shifting the balance away from the active, G-protein-coupled, constitutively signalling receptor conformation, but accurate predictions based on ligand structure are so far not possible. Inverse agonist activity is not in direct relationship with receptor affinity, but differences in negative efficacy are rather dependent on differences among antagonists in their relative affinity for resting (uncoupled) receptors vs. spontaneously active (precoupled) receptors [15].

Transfected PC12 cells expressing α_{2B}-AR at relatively high density provide a useful approach to screen inverse agonists for this class of receptors. Our results further indicate that receptor density undoubtedly plays an important role in influencing the apparent pharmacological profiles of different classes of ligands in recombinant test systems, not only of partial agonists as predicted by classical receptor theory, but also of antagonists and full agonists.

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