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α_{2C}-Adrenoceptor blockade by clozapine and other antipsychotic drugs

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Abstract

The noradrenergic system may play a role in antipsychotic modulation of schizophrenia symptoms. Therefore, the antagonistic potencies of the antipsychotics clozapine, chlorpromazine, risperidone, olanzapine, haloperidol, quetiapine, ziprasidone, iloperidone and aripiprazole were quantified using cell lines expressing the recombinant human α_{2C}-adrenoceptor, α_{2A}-adrenoceptor, or dopamine D_{2L} receptor. The α_2-adrenoceptor antagonists, yohimbine and idazoxan, were also tested. Alterations in cAMP were measured as changes in luminescence. In the α_{2A}-adrenoceptor cell line, the agonist 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK14,304) induced a concentration-dependent increase in luminescence. In cell lines expressing α_{2C} and D_{2L} receptors, agonists induced a concentration-dependent reduction in luminescence. Yohimbine and idazoxan were the most potent α_{2A}-adrenoceptor antagonists, yohimbine and iloperidone were the most potent α_{2C}-adrenoceptor antagonists, and haloperidol and olanzapine were the most potent dopamine D_2 receptor antagonists. Clozapine had the highest α_{2C}/D_2 selectivity, and iloperidone the highest α_{2C}/α_{2A} ratio. It is hypothesised that α_{2C}-adrenoceptor blockade contributes to improvement of cognitive function.

Keywords: α_2-Adrenoceptor antagonist; Antipsychotic; Functional affinity; Cognitive function; Clozapine; Iloperidone

1. Introduction

The clinical potency of antipsychotic compounds is related to the affinity for dopamine D_2 receptors (Seeman et al., 1976; Creese et al., 1976; Schotte et al., 1996; Kapur et al., 2000). Although the antipsychotic compounds display rather equivalent efficacy against positive symptoms, they differ with respect to improvement of cognitive dysfunction (King, 1994; Sharma, 1999) and regarding the induction of extrapyramidal side effects (Leucht et al., 1999). Reduction of D_2 affinity seems to be a mechanism to decrease liability for extrapyramidal side effects (Kapur and Seeman, 2001). Improvement or deterioration of cognitive function induced by antipsychotic treatment is probably related to auxiliary receptor activities (Keefe et al., 1999; Meltzer and McGurk, 1999). The more recently developed polyvalent antipsychotic medications affect several neurotransmitter systems, including the cholinergic, noradrenergic, serotoninergic, histaminergic and dopaminergic systems (Schotte et al., 1996). Clozapine, with its unique efficacy in severely treatment-resistant patients (Kane et al., 1988), is also unique among the polyvalent antipsychotics as it has a profound effect on central and peripheral noradrenaline turnover (Bürki et al., 1974; McMillen and Shore, 1978; Pickar et al., 1992; Breier et al., 1994; Elman et al., 1999). Findings from animal experiments suggest that noradrenaline may play a modulating role in working memory by regulating distraction by irrelevant stimuli during task performance (Arnsten et al., 1996; Friedman et al., 1999). Consistent with this notion, clozapine improved attention, reaction time and accuracy in neurocognitive tasks in patients with schizophrenia (Lee et al., 1999; Galletly et al., 2000; Purdon et al., 2001). Enhancement of noradrenaline turnover (Starke et al., 1989) and improvement of cognitive function (Arnsten et al., 1996; Coull, 1994) are mediated by the α_2-adrenoceptor subtype. It should be noted, however, that the α_2-adrenoceptor is further divided into α_{2A}, α_{2B} and α_{2C} receptor subtypes (Bylund et al., 1994), each with a distinct distribution and function in the brain (Scheinin et al., 1994). The α_{2A} and α_{2C} are the predominant subtypes in the brain (Scheinin et al., 1994).

The aim of this study was to characterise the intrinsic activity and potency of binding for clozapine and other antipsychotic compounds at the human α_{2A}- and α_{2C}-adre-

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noceptors. Since the potency of antipsychotics is related to dopamine D_2 receptor occupancy, with approximately $\geq 50\%$ occupancy required for adequate control of positive symptoms (Nordström et al., 1993; Kapur et al., 2000), the intrinsic activity and potency of antipsychotics at the human D_{2L} receptors were tested as well. As test systems, cell lines expressing recombinant α_{2A}, α_{2C} and D_{2L} receptors were used. As the α_2 and D_2 receptors are coupled to adenylate cyclase, changes in luminescence induced by cAMP-response element (CRE) were taken as functional read-outs.

2. Materials and methods

2.1. Materials

Standard chemicals were obtained from Sigma (Buchs, Switzerland) unless specified otherwise. Clozapine, chlorpromazine, olanzapine, quetiapine, risperidone, aripiprazole, iloperidone, ziprasidone (all synthesised at Novartis Pharma), yohimbine and haloperidol were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 10 mM and diluted further with distilled water. Noradrenaline, dopamine, 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline (UK14,304) and coenzyme-A lithium salt were dissolved in distilled water. Forskolin was dissolved in ethanol.

2.2. Cell incubations

2.2.1. α_{2C}-Adrenoceptor

Chinese hamster ovary (CHO) K1 cells stably expressing the human α_{2C}-adrenoceptor were kindly provided by Dr. M. Caron (Duke University). These cells were transfected with the plasmids pCRE-Luci and pcDNA3.1/Hygro(+) (Invitrogen, Groningen, The Netherlands) according to the protocol of Chen and Okayama (1987). The pCRE-Luci plasmid, containing the luciferase coding region under the control of the cytomegalovirus minimal promoter and six CRE motifs, was generated at Novartis Pharma. Fifty hygromycin-resistant clones were assessed for the production of luminescence in response to 10 μM forskolin. Of the 50 cloned cell lines, 6 were positive; the clone with the highest response to forskolin was selected for all subsequent experiments.

The cells were grown in 96-well plates in Ham's F12 medium with 10% foetal calf serum, 2 mM glutamine, 80 μg/ml G418 (Gibco BRL) and 200 μg/ml hygromycin (Gibco BRL) until confluent. The growth medium was then replaced with serum-free medium. Twelve hours later, freshly prepared solutions of forskolin (5 μM) and (a) either antagonist or noradrenaline alone, or (b) a combination of antagonist plus noradrenaline, were added to the cells. The cells were incubated for 4 h in 150 μl of serum-free medium. The concentrations of noradrenaline were 0.1–100,000 nM. The concentrations of the antagonists were as follows: yohimbine (5–100 nM), idazoxan (1000–10,000 nM), iloperidone (50–500 nM), clozapine (100–10,000 nM), risperidone (50–1000 nM), chlorpromazine (100–30,000 nM), olanzapine (2500–10,000 nM), haloperidol (5000–10,000 nM), aripiprazole (1000–10,000 nM), ziprasidone (1000–10,000 nM) and quetiapine (5000–10,000 nM). Luciferase activity was determined as described below.

2.2.2. α_{2A}-Adrenoceptor

CHO K1 cells stably expressing the human α_{2A}-adrenergic receptor were kindly provided by Dr. M. Caron (Duke University, Durham, NC, USA). These cells were transfected with the plasmids pCRE-Luci and pcDNA3.1/Hygro (+) (Invitrogen) according to the protocol of Chen and Okayama (1987). The pCRE-Luci plasmid containing the luciferase coding region under the control of the cytomegalovirus minimal promoter and six CRE motifs was generated at Novartis Pharma. The 110 hygromycin-resistant clones were analysed for the production of luminescence in response to 10 μM forskolin. Of the 110 cloned cell lines, 6 were

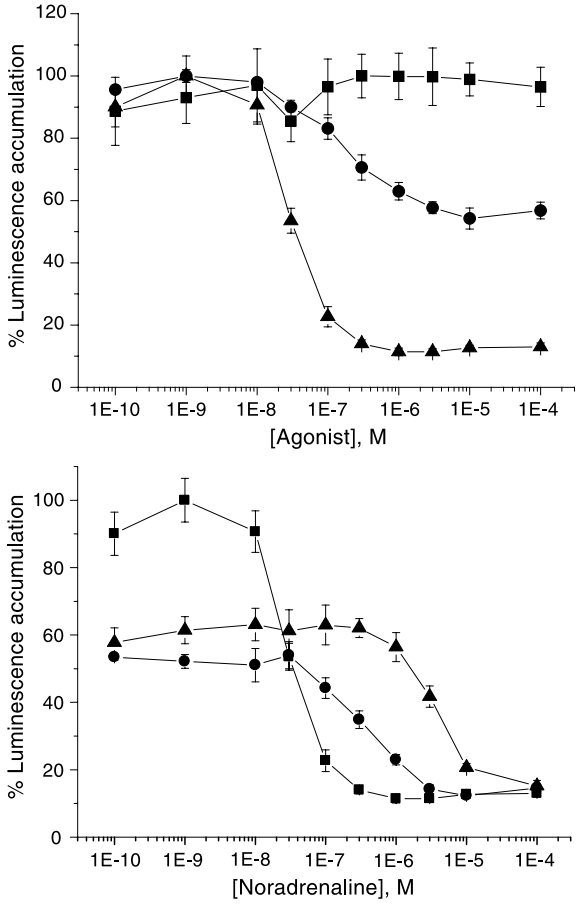

Fig. 1. Top. Typical example of concentration–response curves for accumulation of luminescence at the human α_{2C}-adrenoceptor (■: solvent; ●: idazoxan; ▲: noradrenaline). Idazoxan behaved as a partial agonist in this assay. Bottom. Typical example of concentration–response curve of noradrenaline for accumulation of luminescence in the presence or absence of idazoxan at the human α_{2C}-adrenoceptor (■: solvent; ●: idazoxan 1000 nM; ▲: idazoxan 10,000 nM). In this test, idazoxan, despite partial agonist effect, shifted the concentration–response curve for noradrenaline to the right. Data points represent means +/− standard deviation of a triplicate measurement.

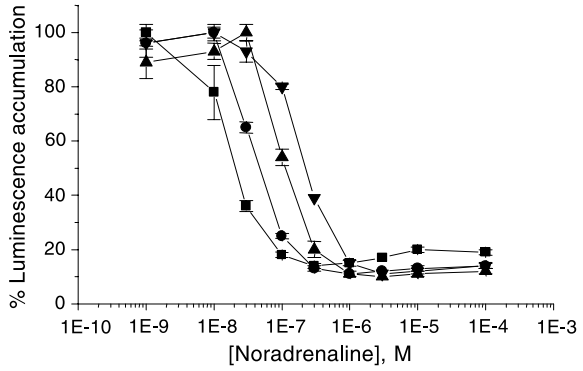

Fig. 2. Typical example of concentration–response curve of noradrenaline for accumulation of luminescence in the presence or absence of clozapine at the human α_{2C}-adrenoceptor (■: solvent; ●: clozapine 100 nM; ▲: clozapine 500 nM; ▼: clozapine 1000 nM). Data points represent means +/− standard deviation of a triplicate measurement.

positive; the clone with the highest response to forskolin was selected for all subsequent experiments.

Cells were grown in 96-well plates in Ham's F12 medium with 10% foetal calf serum, 2 mM glutamine, 80 μg/ml G418 (Gibco BRL) and 200 μg/ml hygromycin (Gibco BRL) until confluent. The growth medium was then replaced by serum-free medium; 12 h later, freshly prepared solution of (a) either antagonist or UK14,304 (1–30,000 nM) alone, or (b) a combination of antagonist and UK14,304 (1–30,000 nM), were added to the cells. The concentrations of the antagonists were as follows: yohimbine (10–100 nM), clozapine (500–5000 nM), iloperidone (250–5000 nM), risperidone (300–1000 nM), quetiapine and chlorpromazine (5000–10,000 nM). The incubation took place at 37 °C for a period of 4 h. Luciferase activity was determined as described below.

2.2.3. D_{2L} receptor

Human embryonal kidney 293 (HEK293) cells stably expressing both the luciferase (CRE-induced) and the human D_{2L} gene were obtained from Knoll (Ludwigshafen, Germany). The cells were grown in 96-well plates in Dulbecco's modified Eagles medium/Ham's F12 medium (1:1), 10% foetal calf serum, 2 mM glutamine and 500 μg/ml G418 (all Gibco BRL). Once confluent, the growth medium was replaced with serum-free medium. Twelve hours later, freshly prepared solutions of forskolin (10 μM) and (a) either antagonist or dopamine alone, or (b) a combination of antagonist plus dopamine, were added to the cells. The cells were incubated at 37 °C for 4 h in 200 μl of serum-free medium. The concentrations of dopamine were 0.1–10,000 nM. The concentrations of the antagonists were as follows: yohimbine (10,000 nM), iloperidone (50–500 nM), clozapine (750–5000 nM), risperidone (10–75 nM), olanzapine (10–100 nM), haloperidol (10–100 nM), chlorpromazine (30–100 nM), ziprasidone (100–1000 nM) and quetiapine (2500–10,000 nM). Aripiprazole was incubated in concentrations of 0.01–10000 nM. Luciferase activity was determined as described below.

2.3. Luciferase assay

The cells were lysed for 15 min at room temperature with 25 μl of lysis buffer [25 mM Tris phosphate, pH 7.8, 2 mM 1,4-dithiothreitol, 2 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), 10% glycerol, 1% Triton X-100]. Luciferase activity was measured in 50 μl of luciferase assay buffer (20 mM tricine, 1.07 mM $MgCO_3 \cdot 5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM 1,4-dithiothreitol, 270 μM coenzyme-A lithium salt, 470 μM D-luciferin sodium, 530 μM ATP, pH 7.8) using an Anthos Lucy 1 luminometer (Anthos Labtech Instruments, Salzburg, Austria). The emitted light units were integrated for a period of 1s. Each data point was determined in triplicate. The luminescence data points are considered to reflect cAMP levels.

2.4. Analysis of data

Agonist-induced percentage inhibition of luminescence in the presence of forskolin was calculated using the logistic equation $f(x) = E_{max}/(1 + IC_{50}/x)$, where x is the concentration of agonist and $f(x)$ the percentage inhibition, using the Microcal Origin® software package. The antagonist K_B value was

Table 1
Antagonist pK_B values for human α_{2C}-adrenoceptor, α_{2A}-adrenoceptor and D_{2L} receptor

Compound	pK_B	S.E.M.	*N*	pK_B	S.E.M.	*N*	pK_B	S.E.M.	*N*	Ratio	Ratio
	α_{2C}			α_{2A}			D_{2L}			α_{2C}/α_{2A}	α_{2C}/D_2
Yohimbine	8.50	0.02	05	8.78	0.03	06	5.37	0.10	03	0.5	1349
Idazoxan	7.10	0.03	08	7.48	0.05	06	<5		02	0.4	>125
Clozapine	7.27	0.04	06	6.75	0.02	05	6.18	0.07	10	3	12
Iloperidone	7.83	0.06	15	6.74	0.05	08	7.56	0.04	13	12	2
Quetiapine	6.10	0.02	07	5.15	0.13	05	5.95	0.05	07	9	1
Risperidone	7.78	0.04	06	7.22	0.04	05	8.14	0.06	06	4	0.4
Ziprasidone	6.16	0.03	08				7.17	0.03	05		0.1
Chlorpromazine	5.85	0.06	08	5.38	0.03	05	7.82	0.05	08	3	0.01
Olanzapine	5.97	0.04	07				8.36	0.06	05		0.004
Haloperidol	5.46	0.01	05				8.73	0.06	05		0.0005
Aripiprazole	6.01	0.03	06				*				

* Agonist $pIC_{50} = 8.19 \pm 0.34$; $N = 4$.

calculated from the formula K_B=[B]/(CR − 1), where [B] is the concentration of antagonist and CR is the agonist IC_{50} concentration ratio with and without antagonist (Furchgott, 1972). Although we tested whether the blockade of the agonist-induced responses was concentration-dependent, it was not the intention to formally investigate the competitive nature of the blockade by means of Schild-plot analyses.

3. Results

3.1. α_{2C}-Adrenoceptor

Noradrenaline caused a concentration-dependent reduction of forskolin-induced cAMP (Figs. 1 and 2). With the exception of idazoxan, none of the compounds induced significant agonist activity in cells transfected with the human α_{2C}-adrenoceptor gene. The intrinsic activity of idazoxan varied strongly from experiment to experiment and ranged from 10% to 50% of the noradrenaline-induced effect. A typical experiment is shown in Fig. 1. Despite intrinsic activity, the potential antagonist properties could be determined. Idazoxan shifted the concentration–response curves of noradrenaline rightward, but concomitantly reduced the initial level of luminescence (an example of an experiment is shown in Fig. 1). Clozapine was a 'silent' antagonist and caused a concentration-dependent shift of the concentration–response curve for noradrenaline for accumulation of luminescence to the right in a parallel fashion (see Fig. 2). Other antipsychotic compounds with potent α_{2C}-adrenoceptor antagonist properties were iloperidone and risperidone (data not shown). Aripiprazole could not be tested at concentrations beyond 5000 nM because of cellular toxicity. The rightward shift in the concentration–response curves was used to calculate K_B values according to the method of Furchgott (1972).

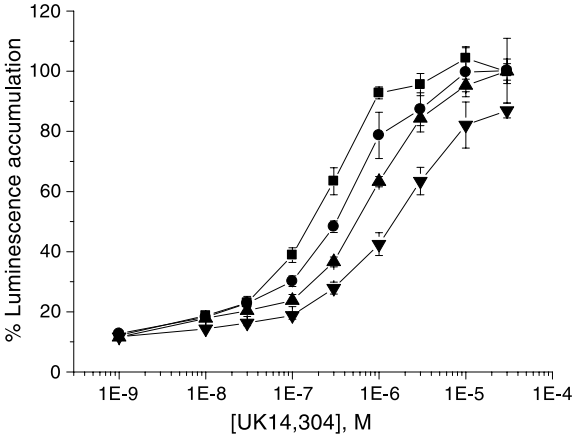

Fig. 3. Typical example of concentration–response curve of UK14,304 for accumulation of luminescence in the presence or absence of idazoxan at the human α_{2A}-adrenoceptor (■: solvent; ●: idazoxan 50 nM; ▲: idazoxan 100 nM; ▼: idazoxan 250 nM). Data points represent means +/− standard deviation of a triplicate measurement.

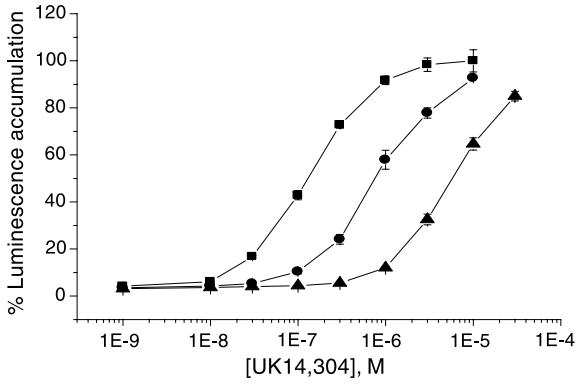

Fig. 4. Typical example of concentration–response curve of UK14,304 for accumulation of luminescence in the presence or absence of clozapine at the human α_{2A}-adrenoceptor (■: solvent; ●: clozapine 1000 nM; ▲: clozapine 5000 nM). Data points represent means +/− standard deviation of a triplicate measurement.

Yohimbine was the most potent α_{2C}-adrenoceptor antagonist [mean (S.E.M.) pK_B value = 8.50 (0.02)], followed by iloperidone [mean (S.E.M.) pK_B value = 7.83 (0.06)] (Table 1). Potency of the other compounds fell in the following order: risperidone>clozapine>idazoxan>ziprasidone>quetiapine>aripiprazole>olanzapine>chlorpromazine>haloperidol.

3.2. α_{2A}-Adrenoceptor

The potency to block the α_{2A}-adrenoceptor was investigated for a selection of compounds that potently interacted with the α_{2C}-adrenoceptor: yohimbine, idazoxan, clozapine, quetiapine, risperidone, chlorpromazine and iloperidone. In the α_{2A}-adrenoceptor expressing cells used for the present experiments, the endogenous agonist, noradrenaline, and the potent α_{2A} receptor agonist, UK14,304, activated, rather than inhibited, adenylate cyclase. None of the test compounds (including idazoxan)

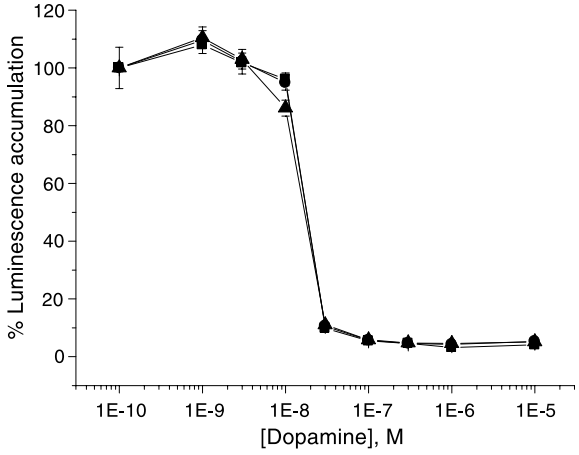

Fig. 5. Typical example of concentration–response curve of dopamine for accumulation of luminescence in the presence or absence of idazoxan at the human D_{2L} receptor (■: solvent; ●: idazoxan 1000 nM; ▲: idazoxan 10,000 nM). Data points represent means +/− standard deviation of a triplicate measurement.

displayed significant intrinsic activity in this cell system. Yohimbine, idazoxan (Fig. 3), clozapine (Fig. 4) and other antipsychotic compounds shifted the concentration–response curves for the effect on luminescence by UK14,304 to the right in a parallel fashion. As in the previous section, K_B values were calculated according to Furchgott (1972). Yohimbine was the most potent α_{2A}-adrenoceptor antagonist, followed by idazoxan, risperidone, clozapine and iloperidone.

3.3. D_{2L} receptor

Consistent with previous studies (Sokoloff and Schwartz, 1995), the functional response to D_{2L} receptor stimulation in HEK293 cells was a reduction in forskolin-induced intracellular cAMP levels. With the exception of aripiprazole, none of the compounds tested had significant agonist activity in cells transfected with the human D_{2L} receptor gene. In the present cell system, aripiprazole was as efficacious as the endogenous agonist, dopamine, but had an approximately 40-fold higher affinity. Due to this full agonist response, a K_B value for aripiprazole could not be established. The remaining antipsychotic compounds, but not the reference α_2-adrenoceptor antagonists, shifted the concentration–response curve for dopamine for accumulation of luminescence to the right in a parallel fashion. Typical experiments are shown for idazoxan (Fig. 5) and clozapine (Fig. 6). These shifted curves were again used to calculate K_B values according to the method of Furchgott (1972).

Haloperidol was the most potent dopamine D_2 receptor antagonist [mean (S.E.M.) pK_B value = 8.73 (0.06)], followed by olanzapine [mean (S.E.M.) pK_B value = 8.36 (0.06)] (Table 1). Potency of the other compounds fell in the following order: risperidone>chlorpromazine>iloperidone>ziprasidone>clozapine>quetiapine>yohimbine>idazoxan.

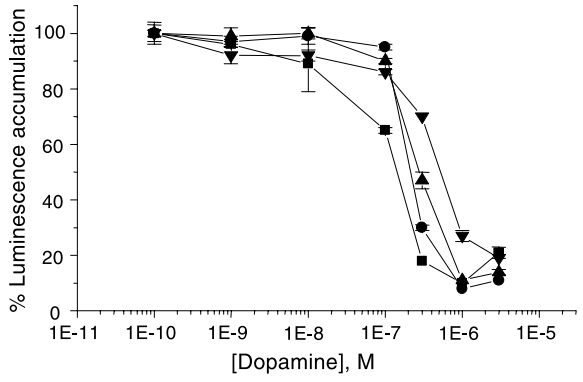

Fig. 6. Typical example of concentration–response curve of dopamine for accumulation of luminescence in the presence or absence of clozapine at the human D_{2L} receptor (■: solvent; ●: clozapine 500 nM; ▲: clozapine 1000 nM; ▼: clozapine 5000 nM). Data points represent means +/− standard deviation of a triplicate measurement.

3.4. α_{2C}/D_2 receptor selectivity

Clozapine was the antipsychotic drug with the highest α_{2C}/D_2 selectivity (α_{2C}/D_2 ratio = 12) followed, in order, by iloperidone (ratio = 2), quetiapine (ratio = 1), risperidone (ratio = 0.4), ziprasidone (ratio = 0.1), chlorpromazine (ratio 0.01) and olanzapine (ratio = 0.004). As might be expected, haloperidol had the lowest α_{2C}/D_2 selectivity (ratio = 0.0005) (Table 1).

3.5. α_{2C}/α_{2A} Receptor selectivity

Both yohimbine and idazoxan were slightly more potent α_{2A} antagonists than α_{2C} antagonists (α_{2C}/α_{2A} ratios = 0.5 and 0.4, respectively). The opposite was true for clozapine, chlorpromazine and risperidone (α_{2C}/α_{2A} ratio = 3–4), quetiapine (ratio 9) and iloperidone (ratio = 12).

4. Discussion

4.1. General considerations

Earlier functional characterisation studies of α_2-adrenoceptors in native tissue have generally reported that agonists result in an inhibition of cAMP production (Bylund et al., 1994). In the present experiments, activation of human α_{2C}-adrenoceptors expressed in CHO cells diminished cAMP levels, which is consistent with the common view that α_2-adrenoceptors couple to G_i/G_o proteins. In contrast, activation of human α_{2A}-adrenoceptors expressed in CHO cells increased cAMP levels. Increases in cAMP levels in response to α_2-adrenoceptor stimulation is not unprecedented in cell lines expressing recombinant α_2-adrenoceptors (Fraser et al., 1989; Eason et al., 1992, 1994).

The functional response to D_{2L} receptor stimulation in HEK293 cells was a reduction in intracellular cAMP levels, which is consistent with published data (Sokoloff and Schwartz, 1995). In each cell line, incubation with antagonists shifted the concentration–cAMP response curves to the right in an approximately parallel manner. This enabled determination of K_B values for antagonist potency. Although we examined whether the blockade of the agonist-induced responses was concentration-dependent, it was not our intention to formally investigate the competitive nature of the blockade by means of Schild-plot analyses.

4.2. Partial agonist responses

Antipsychotic drugs are generally thought to be antagonists at the sites for which they show high affinity in radioligand binding screens. However, there are exceptions: some antipsychotic drugs are known to activate muscarinic receptors (Zeng et al., 1997; Olianas et al., 1999) or serotonin 5-HT_{1A} receptors (Newman-Tancredi et al., 1998). In the cell assay used for the present study, all antipsychotic drugs were

silent α_{2A}- and α_{2C}-adrenoceptor antagonists. With the notable exception of aripiprazole, the antipsychotic compounds also did not stimulate dopamine D_{2L} receptors. In the HEK293 cells used for the present experiments, aripiprazole displayed an intrinsic activity with a magnitude comparable to that of dopamine. This is in line with Lawler et al. (1999), who used CHO and C-6 cells transfected with rat D_{2L} receptors and noted that aripiprazole displayed partial agonist activity with an affinity higher than dopamine. Intrinsic activity depends on the test system and the receptor reserve therein. Therefore, our finding of a higher intrinsic activity is not unusual. The results with aripiprazole, however, emphasise the point that second messenger experiments provide important information in addition to classical radioligand binding screens.

4.3. Comparison with radioligand binding data

The results from the present functional experiments are in general agreement with the radioligand binding study of Schotte et al. (1996). These authors reported that the binding affinity of antipsychotic compounds was always higher for α_{2C}-adrenoceptors than for α_{2A}-adrenoceptors. Clozapine and risperidone were among the compounds with the highest affinity for α_{2C}-adrenoceptors. The authors reported K_i values of clozapine and risperidone for human recombinant α_{2C}-adrenoceptors as 9.1 nM and clearly lower affinities for ziprasidone (77 nM), olanzapine (210 nM), quetiapine (350 nM) and haloperidol (550 nM). This separation was also found in the present functional study. The binding affinity of iloperidone for the human α_{2C}-adrenoceptor was previously determined as (K_i 16.2 nM) (Kalkman et al., 2001). The α_{2C}-adrenoceptor affinity of aripiprazole has thus far not been published. In a paper by Jasper et al. (1998), idazoxan was devoid of intrinsic activity at the human α_{2C}-adrenoceptor. This is at variance with the present results and may be explained by higher receptor reserve in our cellular system. The results of the present functional affinity experiments generally support the radioligand binding data. In addition, they offer important information regarding the effect of each of these compounds on the receptors studied, which aids in understanding and predicting therapeutic profiles of antipsychotic medications.

4.4. Absolute and relative strength of α_2-adrenoceptor blockade

For therapeutic activity against psychoses, antipsychotic drugs are dosed until central dopamine D_2 receptors are occupied by $\geq 50\%$ (Nordström et al., 1993; Kapur et al., 2000). Newer antipsychotic compounds are generally less selective and bind to multiple receptor systems. In order to contribute to the therapeutic profile, such auxiliary activities should occur at doses that produce relevant central dopamine D_2 receptor blockade. In the current context, this means that it is not so much the *absolute* affinity for α_{2A}- and α_{2C}-adrenoceptors that matters, but rather the *relative* affinity vis-à-vis the dopamine D_2 receptor. Ratios for $\alpha_{2C}/D_2 \geq 1$ were obtained for clozapine, iloperidone and quetiapine, whereas an α_{2A}/D_2 ratio ≥ 1 was found for clozapine but not iloperidone. For antipsychotic compounds with an α_{2C}/D_2 ratio < 1, it is unlikely that α_2-blockade will contribute substantially to the clinical profile. Notably, olanzapine—which has a chemical structure very similar to clozapine including a polyvalent radioligand binding profile (especially if normalised to the respective D_2 affinities)—strongly differs from clozapine with regards to the affinity for α_2-adrenoceptors (Schotte et al., 1996). This was also reflected in the present experiments.

Idazoxan was tested in an augmentation study by Litman et al. (1996). The authors investigated the effect of fluphenazine, an older dopamine D_2 receptor-blocking antipsychotic drug, and fluphenazine plus idazoxan in a double-blind trial of 17 patients with schizophrenia or schizoaffective disorder. The addition of the α_2-adrenoceptor antagonist idazoxan resulted in significant improvement in schizophrenia symptoms compared with fluphenazine alone. Thus, there could be significant functional consequence of α_{2C}- and/or α_{2A}-adrenoceptor blockade by novel antipsychotics.

4.5. Role of α_2-adrenoceptor subtypes in cognitive function

The individual role of each of these subtypes on neurocognition is not clear. Working memory deficits in experimental animals and in humans are ameliorated by α_2-adrenoceptor *agonists* like guanfacine or clonidine (Birnbaum et al., 2000; Li et al., 1999; Jäkälä et al., 1999). The observation that guanfacine seems to induce more robust effects than clonidine has been explained by a higher α_{2A}-subtype selectivity (Jäkälä et al., 1999). The preclinical literature about effects of selective α_2-adrenoceptor *antagonists* in cognition tests is confusing. α_2-Adrenoceptor antagonists both improve (Haapalinna et al., 1998; Middleton et al., 1999) and deteriorate cognitive function (Sawaguchi, 1998; McAllister, 2001). Differences in subtype selectivity might provide an explanation. It is conceivable that α_{2A}-adrenoceptor stimulation has a positive effect on cognition, while α_{2A}-adrenoceptor blockade has a negative effect. The opposite could be true for the α_{2C}-adrenoceptor agonists and antagonists, since overexpression of α_{2C}-adrenoceptors in mice impaired their ability to perform spatial and nonspatial cognitive tasks (Björklund et al., 1999). The novel antipsychotic compound iloperidone (Szewczak et al., 1995) could be an interesting candidate for the evaluation of the α_{2C} hypothesis. This compound displays an α_{2C}-adrenoceptor/dopamine D_2 ratio that is closer to that of clozapine than any other compound presently tested. However, iloperidone remains to be studied in cognition tests.

In conclusion, the affinity for α_{2C}-adrenoceptors and dopamine D_2 receptors was investigated in a series of compounds, including yohimbine, idazoxan, and the antipsychotics clozapine and iloperidone. Yohimbine and

iloperidone were the most potent α_{2C}-adrenoceptor antagonists, and haloperidol and olanzapine were the most potent dopamine D_2 receptor antagonists. Clozapine had the highest α_{2C}/D_2 selectivity, followed by iloperidone, quetiapine, risperidone, ziprasidone, olanzapine and haloperidol. The α_{2C}-adrenoceptor blockade of antipsychotic compounds (if occurring at concentrations needed for dopamine D_2 receptor blockade) may contribute to improvements in working memory.

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