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RECEPTOR DENSITY INFLUENCES THE RECRUITMENT BIAS OF ARIPIPRAZOLE AND BREXPIPRAZOLE AT THE DOPAMINE D_{2L} RECEPTOR

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KEYWORDS

antipsychotics, dopamine, functional selectivity, receptor density, schizophrenia, signaling bias

ABSTRACT

Aripiprazole, brexpiprazole, and cariprazine are dopamine D_2 receptor ligands considered as effective and tolerable antipsychotics. Brain imaging studies showed that schizophrenia is characterized by elevated dopamine receptor density, which is exacerbated by antipsychotic treatments. Despite the complexity of translating in vitro studies to human neurobiology, overexpression experiments in transfected cells provide a proof-of-concept model of the influence of receptor density on antipsychotic treatments. Since receptor density was demonstrated to influence the signaling profile of dopaminergic ligands, we hypothesized that high dopamine D_2 receptor expression levels could influence the recruitment of G_{i1} and β-arrestin2 in response to partial agonists used as antipsychotics. A nanoluciferase complementation assay was used to monitor β-arrestin2 and G_{i1} recruitment at the dopamine D_{2L} receptor in response to aripiprazole, brexpiprazole, and cariprazine. This was performed in transfected cells carrying a doxycycline-inducible system allowing to manipulate the expression of the dopamine D_{2L} receptors. Increasing D_{2L} receptor density reoriented aripiprazole's preferential recruitment from G_{i1} to β-arrestin2. With respect to brexpiprazole, which showed inverse agonism for β-arrestin2 recruitment at the lower receptor density tested, inverse agonism for G_{i1} recruitment was observed when tested at a high receptor expression level. At variance, cariprazine evoked a potent partial agonism for β-arrestin2 recruitment only, in all the tested conditions. D_{2L} receptor density appears to shape the recruitment bias of aripiprazole and brexpiprazole, but not cariprazine. This suggests that changes in receptor expression level could qualitatively influence the functional response of partial agonists used in psychiatry.

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1. Introduction

The dopamine D_2 receptor partial agonist aripiprazole is an antipsychotic drug characterized by appreciated efficacy and tolerability.[1,2] The exploration of its pharmacological profile at the dopamine D_2 receptor has revealed its capacity to activate different signaling pathways with distinct efficacies or potencies, a phenomenon referred to as functional selectivity.[3-6] This phenomenon reflects the complex signaling associated with the activation of metabotropic receptors, namely conveyed by G proteins or β-arrestins. While G proteins are mainly dedicated to signal transduction, β-arrestins are also associated with receptor desensitization and internalization and are thus conferred with a multidimensional biochemical role in receptor function. Importantly, β-arrestin signaling in response to dopamine D_2 receptor stimulation is implicated in the dopamine-mediated modulation of locomotion and behavior through the PP2A/PKB/ GSK3β pathway.[7,8]

The examination of the βarrestin2 recruitment at the D_{2L} receptor in response to aripiprazole has yielded inconsistent results. Reports obtained from bioluminescence resonance energy transfer (BRET) approaches or Tango™ protease-based assays designated aripiprazole as an antagonist and as a partial agonist, respectively.[9,10] Hence, the pharmacological profile of aripiprazole at the D_{2L} receptor has been described as vacillating between partial agonism and antagonism according to the transduction pathway that is considered and the receptor density in the experimental setup, adding a further layer of complexity to the proposed mechanism of action of this antipsychotic.[11-16]

Of note, the density of D_{2L} receptors sharply increases in the striatum of medicated schizophrenic patients favoring the occurrence of undesired extrapyramidal symptoms (EPS)[17] and requiring prescribers to switch to more tolerable antipsychotic agents.[18] As receptor density is increased during the pharmacological treatment of schizophrenia, and considering that changes in receptor expression are thought to influence the properties of aripiprazole, we herein hypothesized that manipulating D_{2L} receptor density could alter the functional selectivity of this antipsychotic drug. In order to limit experimental biases, we have monitored the recruitment of G_{i1} or β-arrestin2 proteins at the D_{2L} receptor using a unique approach, based on the nano-luciferase complementation technology. This biosensor, characterized by enhanced reversibility and sensitivity when compared to other assays detecting protein-protein interactions, was applied in a cellular model enabling the manipulation of D_{2L} receptor expression by a tetracycline-inducible transcription promoter. Our study was extended to brexpiprazole and cariprazine, two antipsychotics recently adopted in clinical settings.[19] Since these compounds share a putative partial agonism at the dopamine D_2 receptor with aripiprazole, we hypothesized that manipulating receptor density could also influence the pharmacological profile of these drugs, whose functional selectivity remains unraveled.

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2. Materials and methods

2.1. MATERIALS

Dopamine, aripiprazole, Triton X, poly-L-lysine, paraformaldehyde (PFA), X-tremeGene™ HP DNA transfection reagent, and DMSO were from Sigma-Aldrich (Diegem, Belgium). L-glutamine, penicillin/streptomycin, lipofectamine, and trypsin-EDTA were from Thermo Fisher Scientific (Waltham, MA). Cell culture medium (Dulbecco's modified Eagle medium), G418 (50 mg/ml stock), and Hygromycin (50 mg/ml stock) were from Invitrogen (Merelbeke, Belgium). Brexpiprazole was from MedChemExpress LLC (Monmouth Junction, NJ). Cariprazine was from Selleck Chemicals (Houston, TX). Six-well and 96-well plates were from Greiner Bio-One (Wemmel, Belgium). Fetal bovine serum (FBS) was from Biowest (Riverside, MO). Doxycycline was from TakaraBio (Kusatsu, Japan). Coelenterazine-h was from Regis Technologies (Morton Grove, IL).

2.2. MOLECULAR CLONING

The constructs employed in this experimental report (D_{2L}-NP, G_{i1}-LgBiT, and β-arrestin2-LgBiT) were developed by Laschet et al.[20] The D_{2L}-NP construct was obtained by cloning the D_{2L} receptor sequence, flanked at its N-terminus by a signal sequence and a Flag epitope in a pcDNA3.1 + plasmid. In addition, a flexible linker, followed by the small subunit of the Nanoluciferase enzyme, referred to as natural peptide (NP) was added at its C-terminus. Similarly, the sequence encoding for G_{i1} and β-arrestin2 proteins were cloned into the pNBe3 vector (Promega Corporation, Madison, WI, USA), with the addition of a flexible linker (SGGGGS) and the large subunit of the NanoLuciferase enzyme (LgBiT) at their N-terminal. The G_{i1} sequence saw the addition of a HA epitope at its C-terminus.

2.3. CELL CULTURE AND TRANSFECTION

Cell culture was performed as described by Ferraiolo et al.[21] A pTRE construct carrying the D_{2L} receptor cDNA sequence was stably transfected using Lipofectamine™ in HeLa Tet-On cells. The selection step was performed with G418 (500 µg/ml) and hygro-mycin (100 µg/ml), allowing the obtention of the HeLa Tet-On D_{2L} cell line. A limited basal [^{3}H] spiperone binding in the absence of doxycycline enabled to select the most suitable clonal population for experimental purposes, as outlined by Koener et al.[14] HeLa Tet-On D_{2L} and wild-type HeLa cells were cultivated in a controlled environment (37C°, humidified, 5% CO_2 incubator), and in Dulbecco's modified Eagle medium supplemented with 1% l-glutamine, 1% penicillin/strep-tomycin, 10% FBS. G418 (250 µg/ml) and hygromycin (50 µg/ml) were added to the medium of HeLa Tet-On D_{2L} cells. Cells were seeded in six-well plates at a density of 2 million cells/well 48 h before nanoluciferase complementation experiments in serum- and antibiotic- free medium. Cells were co-transfected with the D_{2L}-NP and either the G_{i1}-LgBiT or the β-arrestin2-LgBiT plasmids 4 h after plating, and at a 1:1 mass ratio. The co-transfection was performed using the X-tremeGene™ HP DNA transfection reagent, employing a reagent/DNA ratio of 3:1, according to the manufacturer's recommendations. Culture medium was

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replaced after 24 h, with a 10% FBS-enriched medium. When needed, cells were exposed to 3 µg/ml doxycycline for 24 h to induce the expression of the D_{2L} receptor.

2.4. NANOLUCIFERASE COMPLEMENTATION ASSAY FOR THE DETECTION OF G_{i1} OR B-ARRESTIN2 RECRUITMENT AT THE D_{2L} RECEPTOR

Nanoluciferase complementation assays were performed as described by Ferraiolo et al.[21] HeLa Tet-On D_{2L} cells exposed to doxycycline or to a vehicle solution were harvested 48 h after the co-transfection of the cells. They were then resuspended in Hank's balanced salt solution (HBSS) (NaCl 120-mM, KCl 5.4-mM, $MgSO_4$ 0.8-mM, and HEPES 10 mM) supplemented with 0.05-mM $Na_2S_2O_5$, added with the purpose of limiting the oxidation of dopamine in the buffer. Thereafter, cells were seeded in 96-well plates, in a volume of 80 µL at a density of 50 000 cells/well; 10 µl of ligands (at the indicated concentrations) or vehicle solution (HBSS, 8% DMSO) were then added, together with 10 µL of a 10 µM coelenterazine-h solution (Regis Technologies, Morton Grove, IL). After 15 min of incubation, the Nanoluciferase bioluminescent signal was measured with a Topcount® luminescence counter (Perkin Elmer, Waltham, MA).

2.5. DATA ANALYSIS

Bioluminescent readouts were expressed in relative light units (RLUs) as means ± SEM and normalized to the basal signal (cells exposed to vehicle) to correct for variations in receptor expression. Data points represent the mean ± SEM of five independent experiments, performed in triplicates. The efficacy (Emax, expressed as a percentage of response above basal) and potency (pEC_{50}) of the tested compounds was obtained through the non-linear analyses of sigmoidal concentration-response curves.

The Graph Pad Prism software (version 5.03; GraphPad Software, CA) was utilized for performing statistical analyses. After verification of normality, we ran unpaired, two-tailed Student's t tests when comparing two data sets, while one-way ANOVA tests were conducted when comparing more than two data sets. Statistical differences from control conditions were assessed by applying Dunnett's post hoc test.

3. Results

3.1. EXAMINATION OF THE RECRUITMENT BIAS OF ARIPIPRAZOLE, BREXPIPRAZOLE, AND CARIPRAZINE

The controlled overexpression of D_{2L} receptors was achieved using engineered HeLa cells carrying the sequence encoding the D_{2L} receptor under the control of the Tet-On inducible expression system. This model, referred to as HeLa Tet-On D_{2L} cells, has been previously developed and validated by Koener et al.[14] Of note, the authors already quantified D_{2L} receptor expression levels in HeLa Tet-On

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D_{2L} cells exposed or not to doxycycline through [^{3}H]spiperone binding experiments. In detail, exposing the cells to 3 µg/ml of doxycycline for 24 h resulted in the elevation of [^{3}H] spiperone binding by a factor of 10 when compared to cells treated with a vehicle solution (11 029 ± 834 vs. 1036 ± 65 cpm/mg of protein, respectively).[14]

Nanoluciferase complementation assays aiming at examining the recruitment of G_{i1} and β-arrestin2 at the D_{2L} receptor were performed by taking advantage of the NanoBiT® technology.[22] The co-expression of the D_{2L}-NP construct with either G_{i1}-LgBiT or β-arrestin2-LgBiT in HeLa Tet-On D_{2L} cells allowed to monitor the recruitment of either signal transducers in response to aripiprazole, brexpiprazole, and cariprazine, all reputed as partial agonists of the D_{2L} receptor and clinically used as antipsychotics.

This experimental setup was first run in HeLa Tet-On D_{2L} cells not exposed to doxycycline, and thus without inducing the overexpression of the unlabeled D_{2L} receptor. The examination of the bioluminescent signals from these cells indicated a closely comparable dopamine-evoked recruitment of the examined signal transducers, indicating a balanced profile of the D_2 receptor endogenous ligand (Figure 1a; Table 1). Exposing D2L-NP receptors to aripiprazole led to the potent recruitment of the Gi1-LgBiT construct but failed at eliciting any detectable interaction with the β-arrestin2-LgBiT protein (Figure 1b; Table 1). At variance, treating cells with either brexpiprazole or cariprazine induced no substantial G_{i1} recruitment (Figure 1c,d; Table 1). Nevertheless, luciferase complementation between D_{2L}-NP and β-arrestin2-LgBiT constructs was dose-dependently reduced by brexpiprazole, indicating the inverse agonist features of this compound for the considered signal transduction pathway (Figure 1c; Table 1). Albeit with relatively limited efficacy, cariprazine potently induced β-arrestin2-mediated interactions at the D_{2L}-NP receptor (Figure 1d; Table 1).

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***Figure 1**. Pharmacological profiles of aripiprazole, brexpiprazole, and cariprazine in terms of G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor. Concentration-response curves were plotted by monitoring the bioluminescent signal supported by nanoluciferase complementation, describing the interactions between the D_{2L}-NP receptor and the G_{i1}-LgBiT (G_{i1}) or β-arrestin2-LgBiT (βarr2) constructs after exposing cells to dopamine (a), aripiprazole (b), brexpiprazole (c), and cariprazine (d). Nanoluciferase complementation assays were performed on HeLa Tet-On D_{2L} cells not exposed to doxycycline. Data points represent the mean ± SEM of five independent experiments, performed in triplicates.*

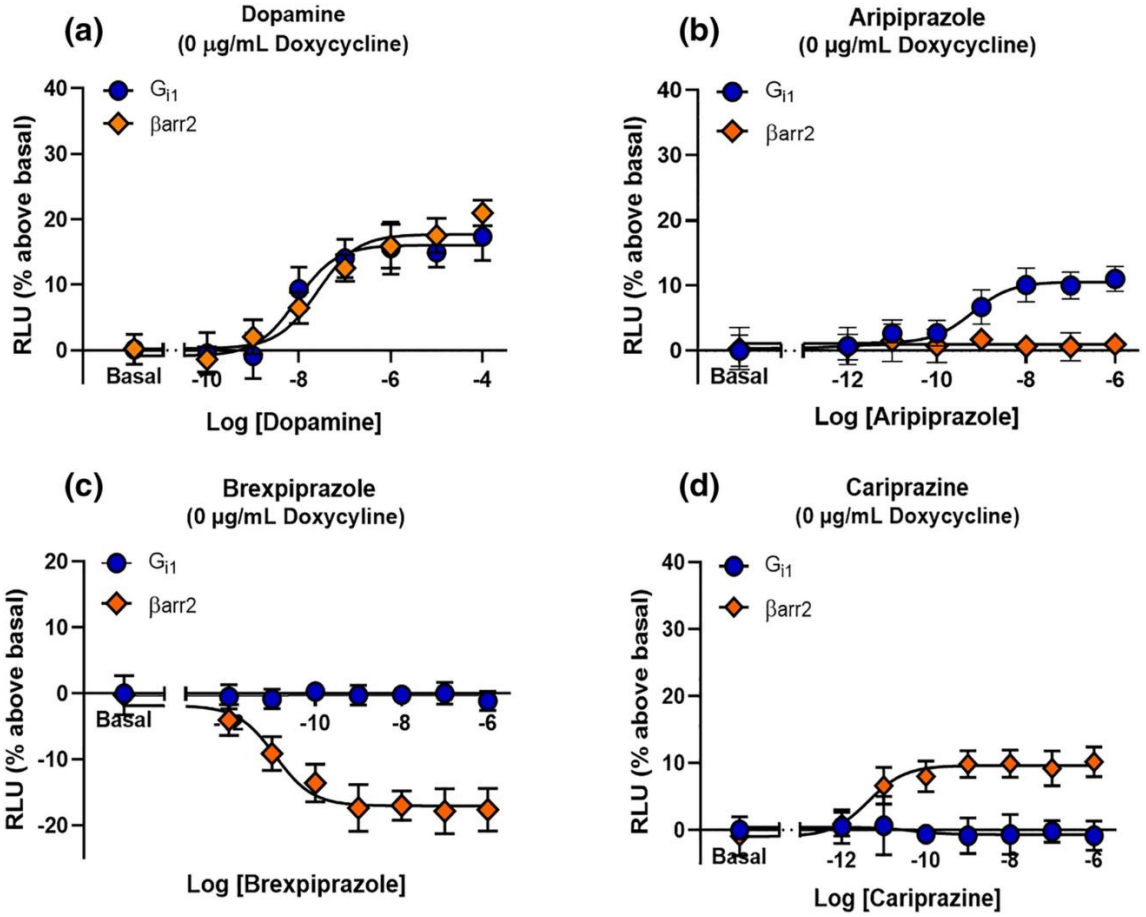

***Table 1**. Summary of efficacy (Emax), potency (pEC_{50}), and recruitment bias of the tested D_{2L} partial agonists obtained from cells not exposed to doxycycline.*

	Emax ± SEM		Student's *t* test (unpaired, two-tailed)		pEC_{50} ± SEM		Student's *t* test (unpaired, two-tailed)		Recruitment bias
Substance	G_{i1}	β-arrestin2	$t_{(8)}$	*p* value	G_{i1}	β-arrestin2	$t_{(8)}$	*p* value	
Dopamine	16.08 ± 1.72	17.71 ± 1.44	0.73	0,49	8.05 ± 0.39	7.60 ± 0.28	0.94	0.38	Balanced
Aripiprazole	10.57 ± 1.38	N/A	N/A		9.2 ± 0.44	N/A	N/A		G_{i1}
Brexpiprazole	N/A	−17.06 ± 1.41	N/A		N/A	10.92 ± 0.35	N/A		Negative β-arrestin2
Cariprazine	N/A	9.6 ± 1.09	N/A		N/A	11.36 ± 0.43	N/A		β-arrestin2

Note: The unpaired, two-tailed Student's t test enabled to calculate the p value of the differences in efficacy and potency measured for G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor, allowing the estimation of recruitment bias.

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3.2. D_{2L} RECEPTOR OVEREXPRESSION REORIENTS THE RECRUITMENT BIAS OF ARIPIPRAZOLE AND BREXPIPRAZOLE, BUT NOT CARIPRAZINE

Growing HeLa Tet-On D_{2L} cells for 24 h in a culture medium supplemented with 3 µg/ml of doxycycline resulted in the overexpression of the D_{2L} receptor (native unmodified sequence). When repeating nanoluciferase complementation experiments in these conditions, dopamine stimulation triggered the preferential recruitment of G_{i1} when compared to β-arrestin2 (Figure 2a; Table 2). In detail, the concentration-response curves obtained with the G_{i1}-LgBiT construct were characterized by efficacy and potency values of significantly higher magnitude than those referring to the β-arrestin2 construct (Figure 2a; Table 2). The stimulation of the D_{2L}-NP receptor with aripiprazole resulted in the recruitment of both G_{i1}-LgBiT and β-arrestin2-LgBiT constructs with comparable efficacies, but with a significantly higher potency when considering β-arrestin2-LgBiT (Figure 2b; Table 2). As such, in experimental conditions characterized by an elevated D_{2L} receptor density, aripiprazole exhibited a β-arrestin2 recruitment bias. Of note, brexpiprazole failed at eliciting any significant β-arrestin2 recruitment at the D_{2L}-NP receptor, while behaving as an inverse agonist when considering G_{i1}-recruitment (Figure 2c; Table 2). Comparable to what was observed in cells not exposed to doxycycline, cariprazine presented a potent partial agonism for β-arrestin2 recruitment, while causing no significant increase in luciferase recombination when testing the G_{i1}-LgBiT construct (Figure 2d; Table 2).

The overall effect of receptor density on the recruitment pattern for the tested compounds is summarized in Figure 3, integrating the data from Figures 1 and 2 in a ligand-oriented perspective. D2L receptor overexpression appeared to influence both dopamine- and brexpiprazole-evoked Gi1 recruitment, promoting the increased Emax of dopamine response (Figure 3a) and revealing the inverse agonism of brexpiprazole (Figure 3c). On the other hand, the potent partial agonism of aripiprazole and the antagonism of cariprazine were not impacted by variations in D_{2L} receptor density (Figure 3b,d). Inducing receptor overexpression using 3 µg/ml of doxycycline did not impact on the β-arrestin2 recruitment at the D2L receptor caused by dopamine or cariprazine stimulation (Figure 3e,h). Nevertheless, a high receptor density was required to detect the partial agonism of aripiprazole for β-arrestin2 recruitment (Figure 3f). Of note, in conditions characterized by a higher receptor density, the inverse agonism of brexpiprazole could not be evidenced (Figure 3g).

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***Figure 2**. Pharmacological profiles of aripiprazole, brexpiprazole, and cariprazine in terms of G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor after incubating cells in the presence of 3 µg/ml of doxycycline. Concentration-response curves were plotted by monitoring the bioluminescent signal supported by nanoluciferase complementation, describing the interactions between the D_{2L}-NP receptor and the G_{i1}-LgBiT (G_{i1}) or β-arrestin2-LgBiT (βarr2) constructs after exposing cells to dopamine (a), aripiprazole (b), brexpiprazole (c), and cariprazine (d). Nanoluciferase complementation assays were performed on HeLa Tet-On D2L cells exposed for 24 h to 3 µg/ml of doxycycline. Data points represent the mean ± SEM of five independent experiments, performed in triplicates.*

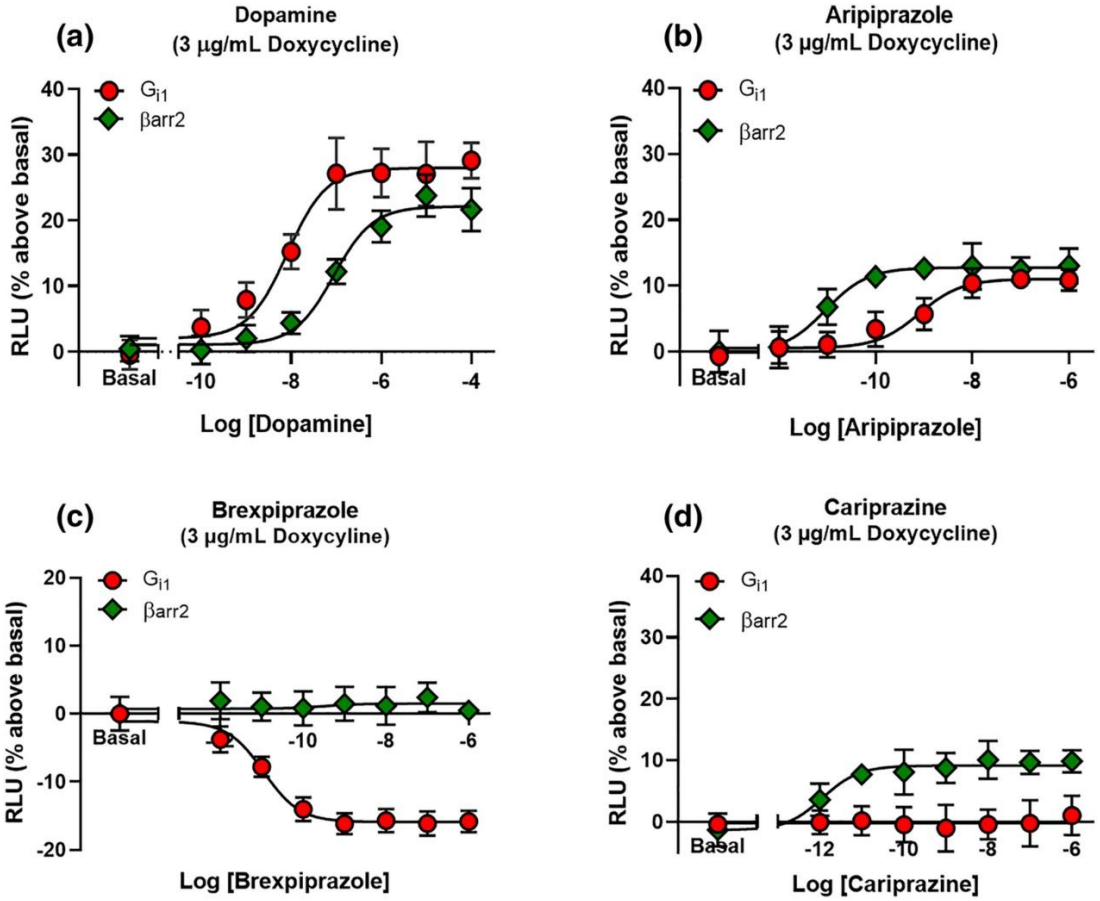

***Table 2**. Summary of efficacy (Emax), potency (pEC_{50}), and recruitment bias of the tested D_{2L} partial agonists after exposing cells to 3 µg/ml of doxycycline.*

	Emax ± SEM		Student's *t* test (unpaired, two-tailed)		pEC_{50} ± SEM		Student's *t* test (unpaired, two-tailed)		
Substance	G_{i1}	β-arrestin2	$t_{(8)}$	*p* value	G_{i1}	β-arrestin2	$t_{(8)}$	*p* value	Recruitment bias
Dopamine	27.98 ± 1.92	22.12 ± 1.59	2.35	0,046	8.08 ± 0.23	7.05 ± 0.20	3.379	0.0097	G_{i1}
Aripiprazole	11.02 ± 1.32	12.76 ± 1.57	0.86	0.42	9.2 ± 0.44	11.03 ± 0.46	2.87	0.021	β-arrestin2
Brexpiprazole	−15.89 ± 0.77	N/A	N/A		10.95 ± 0.21	N/A	N/A		Negative G_{i1}
Cariprazine	N/A	9.13 ± 1.23	N/A		N/A	11.93 ± 0.41	N/A		β-arrestin2

Note: The unpaired, two-tailed Student's t test enabled to calculate the p-value of the differences in efficacy and potency measured for Gi1 and β-arrestin2 recruitment at the D2L receptor, allowing the estimation of recruitment bias.

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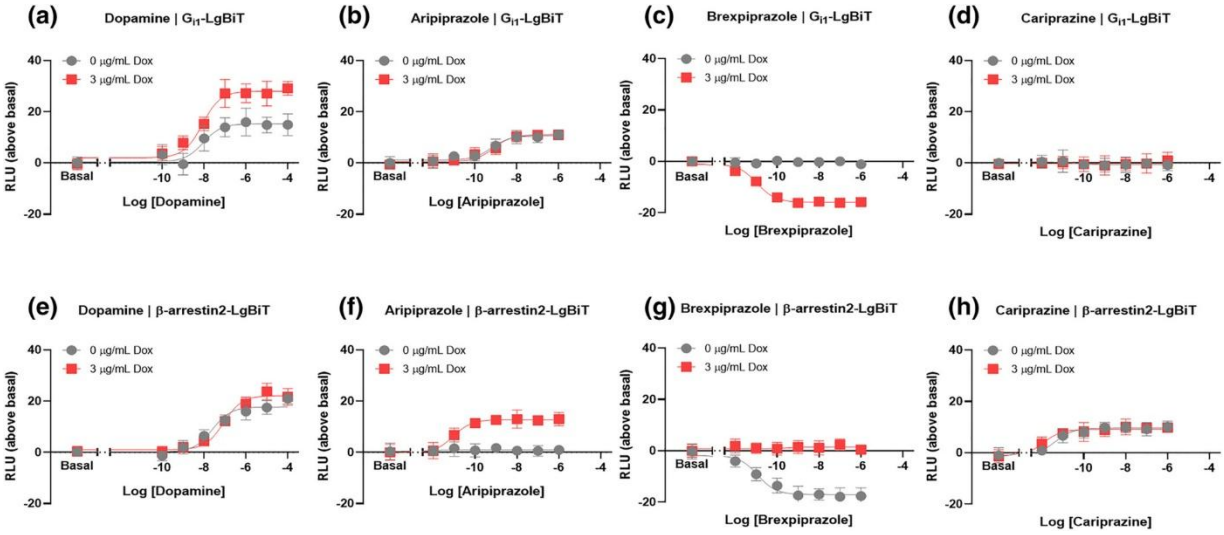
***Figure 3**. Characterization of the effect of D_{2L} receptor overexpression on the pharmacological profile of dopamine, aripiprazole, brexpiprazole, and cariprazine. This figure represents the same dataset of Figures 1 and 2 from a ligand-specific perspective. (a-h) Concentration-response curves were plotted by monitoring the bioluminescent signal supported by nanoluciferase complementation, describing the interactions between the D_{2L}-NP receptor and the G_{i1}-LgBiT (G_{i1}) or β-arrestin2-LgBiT (βarr2). Nanoluciferase complementation assays were performed on HeLa Tet-on D_{2L} cells exposed for 24 h to a vehicle solution (0 µg/ml dox) or to doxycycline (3 µg/ml dox); this latter condition inducing the overexpression of the unmodified, native D_{2L} receptor. Data points represent the mean ± SEM of five independent experiments, performed in triplicates.*



4. Discussion

D_{2L} receptor density has been associated with the development of EPS following prolonged neuroleptic treatment,[17] promoting the risk of psychotic rebounds, agitation, and motor complications in case of antipsychotic switch.[18,23-25] From the molecular standpoint, receptor density has been described as driving the biochemical properties of dopaminergic ligands such as aripiprazole.[14,26] Given the documented functional selectivity of aripiprazole,[6] we hypothesized that receptor density could contribute to determining the recruitment pattern of signal transducers at the D_{2L} receptors. Protein-protein interactions between the D_{2L} receptor and G_{i1} or βarrestin2 were herein examined using a dedicated nanoluciferase assay based on enzymatic complementation. Characterized by an elevated degree of reversibility—contrarily to the majority of protein complementation assays (PCAs)—and higher sensitivity than BRET,[20,22,27] this nanoluciferase complementation assay enabled us to accurately appraise the recruitment bias of aripiprazole, brexpiprazole, and cariprazine. Considering the results obtained from brexpiprazole, such reversibility feature appears as being required to appreciate inverse agonism, which is commonly observed at G protein coupled receptors. Moreover, the use of a single receptor construct in this experimental paradigm markedly limited the influence of system bias on data interpretation.

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Importantly, the experimental paradigm consisted in carrying out the nanoluciferase complementation experiments in a cellular model combining the biosensor with a tetracycline-inducible system controlling the expression of a native form of the D_{2L} receptor, devoid of any luciferase fragment. In such a way, we were able to increase receptor density without changing the expression of the biosensor partners.

The analysis of the recruitment bias of the tested compounds was obtained through direct comparison of their pharmacological parameters—namely, Emax and pEC_{50} values—when measuring G_{i1} or βarrestin2 recruitment at the D_{2L} receptor. Hence, the mathematical computation commonly adopted for examining ligand bias relying on the calculation of transduction coefficients[28,29] could not be exploited in the present study, as this approach yields cumbersome interpretation when applied to antagonism and inverse agonism.[30] Increasing D_{2L} receptor density shifted the recruitment bias of aripiprazole from G^{i1} to βarrestin2 while reorientating the inverse agonism of brexpiprazole from βarrestin2 to G_{i1} recruitment. At variance, a potent partial agonism for βarrestin2 recruitment was preserved for cariprazine in all the tested experimental conditions.

The present data, highlighting the influence of receptor density on the pharmacological profile of aripiprazole, provide a reconciling framework for the conflicting observations obtained in in vitro[9,10] and in vivo studies.[25,31,32] Moreover, our experimental paradigm enabled us to recapitulate the lack of G protein activation by cariprazine[33] together with its potent partial agonism for β-arrestin2 recruitment.[34] Of note, such recruitment bias appeared as independent of receptor density. Surprisingly, despite the fact that brexpiprazole is commonly catalogued as a partial agonist of the D_{2L} receptor, we failed at evidencing any partial agonism of brexpiprazole at the D_{2L} receptor, and so for both G_{i1} and β-arrestin2 recruitment. To the best of our knowledge, we here provide the first observation for the capacity of brexpiprazole to inhibit the constitutive activity of the D_{2L} receptors. However, further research, with heterologous assays detecting the activation of downstream signaling readouts, might be required to validate this observation. Indeed, brexpiprazole has been documented as a partial agonist when monitoring the reduction in forskolin-induced cAMP accumulation, and so with a considerably lower intrinsic activity when compared to aripiprazole.[35] Experimental approaches developed to follow the production of second messengers are frequently designed to amplify signals, facilitating the detection of weak partial agonism. However, this hampers the observation of inverse agonism. One could therefore speculate that the inverse agonism of brexpiprazole (for a given receptor density) might have been confounded with pure antagonism due to the intrinsic amplification of biochemical assays. In this context, it is noteworthy that the majority of antipsychotics commonly considered as D_2 receptor antagonists indeed behave as inverse agonists.[36,37]

Of note, our observations indicate that D_{2L} receptor density impacts the pharmacological profiles of dopaminergic compounds in a ligand-specific way, recapitulating precedent findings on antiparkinsonian agents by our group.[21] How receptor density influences ligand-induced recruitment of signal partners remains to be explored and may involve several molecular or cellular mechanisms. For instance, high receptor density might cause an alteration of the receptor/transducer stoichiometry, reshaping the cellular response to pharmacological agents.

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Indeed, in our experimental paradigm, it would be possible that both native D_{2L} receptors and transfected D_{2L}-NP receptors might compete for the same pool of signal transducers, both endogenous or exogenous (these latter tagged with a LgBiT fragment and determining the measured response). Such competition dynamic occurs to a more limited extent in conditions of lower receptor density, and might therefore identify receptor density as a modulatory factor affect-ing the response to receptor ligands. Moreover, we recently evidenced that increasing D_{2L} receptor density promotes D_{2L} homodimerization, an effect that was inhibited by several antipsychotics, including aripiprazole, brexpiprazole, and cariprazine.[38] Previous studies have shown that D_{2L} homodimers are more efficient signaling units than receptor monomers.[39] Beside receptor/transducer stoichiometry and binding kinetic, receptor density and dimerization would add to the list of pharmacodynamic drivers of the signaling footprint of ligands.[4,40] Of note, this list should also be complemented with biochemical factors not specific to the receptor itself, but of its environment. Indeed, it is relevant to note that dopamine receptors are not homogenously distributed across the cell membrane but are mainly located in membrane microdomains determined by lipid rafts.[41] Hence, it is possible that increased D_{2L} receptor density might have an impact on receptor organization and distribution across such defined membrane compartments. Given the importance of lipid rafts in the determination of dopamine receptor signaling,[41,42] further research should integrate their existence in mechanistic models.

5. Conclusion

In summary, our investigation showed that the D_{2L} receptor density could significantly shape the recruitment bias of aripiprazole and brexpiprazole, but not cariprazine. Given the complexity of the pharmacological treatment of schizophrenia, these observations may pave the way for further studies aiming at better understanding D_{2L} receptor signaling.

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