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Affinity of cyamemazine, an anxiolytic antipsychotic drug, for human recombinant dopamine vs. serotonin receptor subtypes

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Abstract

Animal studies indicate that the anxiolytic properties of the antipsychotic agent cyamemazine may result from blockade of serotonin 5-HT_{2C} receptors and to a lesser extent from blockade of serotonin 5-HT_3 receptors. Here, we used human recombinant receptors to determine the relative affinity of cyamemazine for serotonin and dopamine receptor subtypes. In addition, cyamemazine was tested in other brain receptor types and subtypes which are considered to mediate central nervous systems effects of drugs. Hence, cyamemazine affinity was determined in human recombinant receptors expressed in CHO cells (hD_2, hD_3, and $hD_{4.4}$ receptors, $h5$-HT_{1A}, $h5$-HT_{2A}, $h5$-HT_{2C}, and $h5$-HT_7, and hM_1, hM_2, hM_3, hM_4, and hM_5 receptors), L-cells (hD_1 receptor), and HEK-293 cells ($h5$-HT_3 receptors) or natively present in N1E-115 cells (5-HT_3 receptors) or in rat cerebral cortex (non-specific α_1- and α_2-adrenoceptors, $GABA_A$ and $GABA_B$ receptors, H_3 histamine receptors), and guinea-pig cerebellum (H_1 central and H_2 histamine receptors) membranes. Similarly to atypical antipsychotics, cyamemazine exhibited high affinity for: (i) $h5$-HT_{2A} receptors ($K_i = 1.5 \pm 0.7$ nM, mean ± SEM, N = 3) and this was four times higher than for hD_2 receptors ($K_i = 5.8 \pm 0.8$ nM), (ii) $h5$-HT_{2C} receptors ($K_i = 11.8 \pm 2.2$ nM), and (iii) 5-HT_7 receptors ($K_i = 22$ nM). Conversely, cyamemazine exhibited very low affinity for $h5$-HT_3 receptors ($K_i = 2.9 \pm 0.4$ μM). In conclusion, similarly to atypical antipsychotic agents, cyamemazine, possesses high affinity for $h5$-HT_{2A}, $h5$-HT_{2C}, and $h5$-HT_7 receptors, a feature which can explain its low propensity to cause extrapyramidal adverse reactions in clinical practice. The high affinity for $h5$-HT_{2C} receptors, but not for $h5$-HT_3 receptors, can account for the anxiolytic activity of cyamemazine in human subjects.

Keywords: Cyamemazine; Affinity; Radioligand binding; Recombinant human receptors; Guinea-pig cerebellum; Rat cerebral receptors

1. Introduction

Cyamemazine is a phenothiazine derivative, initially introduced in clinical practice as an antipsychotic agent because of its dopamine D_2 receptor antagonistic activity. However, ample clinical experience indicates that this drug is also useful for the successful treatment of anxiety [1,2] and is efficient in schizophrenic or depressed patients with suicidal tendency. Animal studies showed that: (i) cyamemazine potently antagonizes the effects produced by 5-HT_3 and 5-HT_{2C} serotonin receptor stimulation [3], (ii) cyamemazine is anxiolytic on the elevated plus-maze and light/dark paradigm in mice [4], and (iii) blockade of serotonin 5-HT_{2C} receptors and to a lesser extent 5-HT_3 receptors can mediate the anxiolytic activity of cyamemazine [4].

The primary aim of this study was to compare the affinity of cyamemazine for human recombinant serotonin and dopamine receptor subtypes. In addition, cyamemazine was tested in other receptor types and subtypes which are considered as potential sites of action of therapeutic agents against anxiety, psychosis, and/or depression.

2. Materials and methods

Table 1 lists the 22 receptors which are object of this study. In addition, it contains the biological material used

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Abbreviations: nH, Hill coefficient; K_i, binding constant.

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Table 1
List of receptors studied, biological preparations used to study these receptors, reference compounds applied to validate the binding procedure, and bibliographic references describing details of the binding procedures

Receptor	Origin	Reference compound	Incubation	Bibliography
hD$_1$	*h*-recombinant (L-cells)	SCH 23390	60 min/22°	[6]
hD$_2$	*h*-recombinant (CHO cells)	(+)-Butaclamol	60 min/22°	[7]
hD$_3$	*h*-recombinant (CHO cells)	(+)-Butaclamol	60 min/22°	[8]
hD$_{4.4}$	*h*-recombinant (CHO cells)	Clozapine	60 min/22°	[9]
h5-HT$_{1A}$	*h*-recombinant (CHO cells)	8-OH-DPAT	60 min/22°	[10]
h5-HT$_{2A}$	*h*-recombinant (CHO cells)	Ketanserin	15 min/37°	[11]
h5-HT$_{2C}$	*h*-recombinant (CHO cells)	Mesulergine	30 min/37°	[11]
5-HT$_3$	N1E-115 cells	MDL 72222	180 min/4°	[12]
h5-HT$_3$	*h*-recombinant (HEK-293 cells)	MDL 72222	60 min/22°	[13]
h5-HT$_7$	*h*-recombinant (CHO cells)	Serotonin	120 min/22°	[14]
GABA$_A$	Rat cerebral cortex	Muscimol	10 min/4°	[15]
GABA$_B$	Rat cerebral cortex	Baclofen	10 min/22°	[16]
H$_1$ (central)	Guinea-pig cerebellum	Pyrilamine	10 min/22°	[17]
H$_2$	Guinea-pig striatum	Cimetidine	150 min/22°	[18]
H$_3$	Rat cerebral cortex	(*R*)-α-Me-histamine	60 min/22°	[19]
hM$_1$	*h*-recombinant (CHO cells)	Pirenzepine	60 min/22°	[20]
hM$_2$	*h*-recombinant (CHO cells)	Methoctramine	60 min/22°	[20]
hM$_3$	*h*-recombinant (CHO cells)	4-DAMP	60 min/22°	[20]
hM$_4$	*h*-recombinant (CHO cells)	4-DAMP	60 min/22°	[20]
hM$_5$	*h*-recombinant (CHO cells)	4-DAMP	60 min/22°	[20]
α$_1$ (non-selective)	Rat cerebral cortex	Prazosin	60 min/22°	[21]
α$_2$ (non-selective)	Rat cerebral cortex	Yohimbine	30 min/22°	[22]

for carrying out the binding procedures, the reference compounds used to validate the high affinity binding for each of these receptors, the incubation temperature and time used to determine cyamemazine displacing effects and finally literature references where methodological details for each binding procedure can be found.

At the end of each incubation period (Table 1), membrane or cell suspensions were rapidly filtered under vacuum conditions through glass fiber filters (GF/B, Packard or Filtermat A or B, Wallac). These filters were then washed several times with an ice-cold buffer solution using a cell harvester (Packard or Tomtec).

Bound radioactivity was measured with a scintillation counter (Topcount, Packard or Betaplate, Wallac) using a liquid scintillation cocktail (Microscint 0, Packard) or a solid scintillant product (MeltiLex B/HS, Wallac).

Each competition concentration–response curve was determined in duplicate by using eight concentrations of cyamemazine spanning from 0.03 nM to 30–100 μM. These conditions were also used for generating concentration–response curves to reference compounds in order to validate this experimental procedure. Typical displacement curves are represented in Figs. 1 and 2. Experiments with dopamine and serotonin receptor subtypes were repeated twice (N = 3).

2.1. Analysis and expression of results

The specific radioligand binding to the receptors studied is defined as the difference between total binding and the non-specific binding determined in the presence of an excess of unlabeled ligand.

Results are expressed as a percent of control specific binding obtained in the presence of cyamemazine. Mean data (±SEM) are presented in Section 3.

IC_{50} values (concentration causing 50% inhibition of control specific binding) and Hill coefficients (nH; slope of the curve) were determined by an iterative non-linear regression analysis fitting the competition concentration–response curves with a standard sigmoid curve equation.

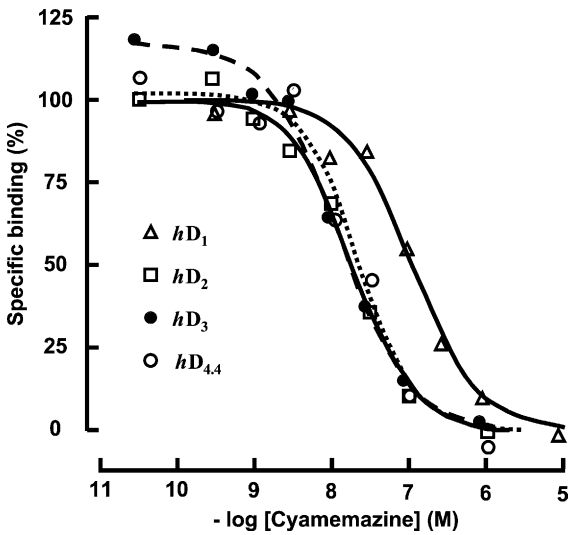

Fig. 1. Effects of cyamemazine on specific binding to human hD$_1$, hD$_2$, hD$_3$, and hD$_{4.4}$ recombinant receptors. The figure represents one typical single experiment of each displacement curve for [^{3}H]SCH 23390 labeling hD$_1$ receptors, (+)-[^{3}H]butaclamol labeling for hD$_2$ and hD$_3$ and [^{3}H]clozapine labeling hD$_{4.4}$ receptors. For mean values (N = 3) see Table 2 and text.

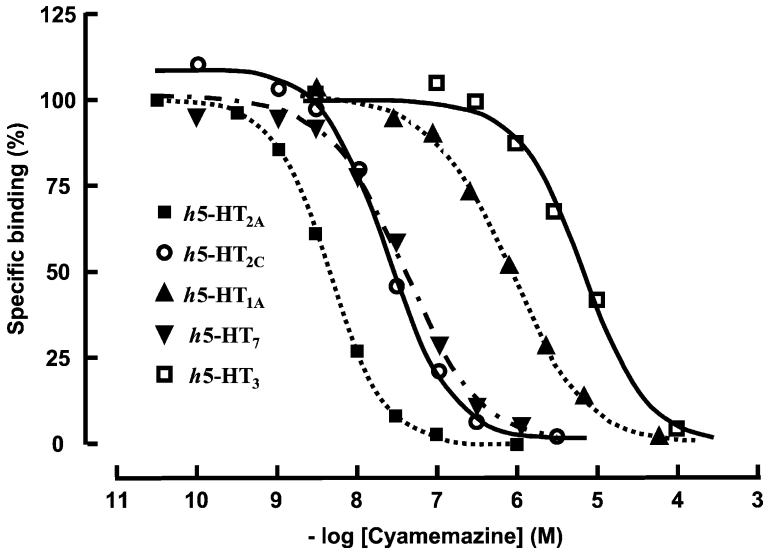

Fig. 2. Effects of cyamemazine on specific binding to various serotoninergic receptors. The figure represents one typical single experiment of each displacement curve for [^{3}H]8-OH-DPTA (labeling $h5$-HT$_{1A}$ receptors), [^{3}H]ketanserin (labeling $h5$-HT$_{2A}$ receptors), [^{3}H]]mesulergine (labeling $h5$-HT$_{2C}$), [^{3}H]MDL 72222 (labeling $h5$-HT$_3$ receptors), and [^{3}H]serotonin (labeling $h5$-HT$_7$ receptors). For mean values (N = 3) see Table 2 and text.

The inhibition constants (K_i) for the binding competition experiments were derived by fitting the experimental data (non-linear least-squares regression analysis) with Cheng Prusoff equation [$K_i = \text{IC}_{50}/(1 + L/K_d)$], where L is the concentration of radiolabeled ligand used for the assay and K_d the affinity of the labeled ligand for the receptor [5].

2.2. Drugs

Cyamemazine tartrate was provided by Aventis Pharma. All reference compounds and radioactive ligands (Tables 1 and 2) used in this investigation were purchased from usual commercial sources.

Table 2
IC_{50} and K_i values for cyamemazine and reference compounds at 22 membrane receptors

Receptor	Cyamemazine			Reference compound			
	IC_{50} (nM)	K_i (nM)	nH		IC_{50} (nM)	K_i (nM)	nH
hD$_1$	12	3.8	1.0	SCH 23390	0.80	0.29	1.2
hD$_2$	16	5.8	1.0	(+)-Butaclamol	8.4	3.0	1.1
hD$_3$	11	2.5	1.1	(+)-Butaclamol	13	2.8	1.3
hD$_{4.4}$	30	5.3	1.2	Clozapine	133	56	1.0
$h5$-HT$_{1A}$	1030	517	0.8	8-OH-DPAT	0.80	0.40	0.8
$h5$-HT$_{2A}$	3.5	1.5	1.4	Ketanserin	2.0	0.90	1.2
$h5$-HT$_{2C}$	36	12	1.1	Mesulergine	2.5	0.81	1.2
5-HT$_3$	201	75	1.0	MDL 72222	22	8.4	1.0
$h5$-HT$_3$	5723	2943	1.1	MDL 72222	10	5.3	1.0
$h5$-HT$_7$	39	22	0.9	Serotonin	0.88	0.49	0.9
GABA$_A$	N.C.	>30 μM	–	Muscimol	29	20	1.1
GABA$_B$	N.C.	>30 μM	–	Baclofen	118	64	0.9
H$_1$ (central)	22	9.3	1.1	Pyrilamine	1.2	0.51	1.0
H$_2$	424	351	0.9	Cimetidine	1390	1150	0.6
H$_3$	37600	21700	1.3	(R)-α-Me-histamine	1.5	0.89	0.9
hM$_1$	16	13	1.1	Pirenzepine	15	12	1.0
hM$_2$	61	42	1.2	Methoctramine	17	12	1.4
hM$_3$	44	32	1.0	4-DAMP	2.6	1.8	1.6
hM$_4$	27	12	0.9	4-DAMP	1.5	0.65	1.2
hM$_5$	59	35	1.1	4-DAMP	1.9	1.1	1.3
α$_1$ (non-selective)	8.5	2.3	1.3	Prazosin	0.85	0.23	1.2
α$_2$ (non-selective)	3060	1320	0.9	Yohimbine	59	26	1.0

Hill coefficients (nH) are also reported. N.C. (non-calculated value) indicates absence of significant displacement at the maximal tested concentration of cyamemazine (100 μM).

3. Results

3.1. Human recombinant dopamine receptor subtypes

The affinity of cyamemazine for four subtypes of human recombinant dopamine receptors expressed in CHO cells was in the nanomolar range (Table 2 and Fig. 1). The highest affinity was for the hD$_3$ receptors ($K_i = 2.5 \pm 0.5$ nM, N = 3), closely followed by the affinity for hD$_1$ receptor ($K_i = 3.8 \pm 0.6$ nM), hD$_2$ ($K_i = 5.8 \pm 0.8$ nM), and hD$_{4.4}$ ($K_i = 5.3 \pm 0.5$ nM) receptors (Table 2 and Fig. 1).

3.2. Human recombinant serotoninergic receptor subtypes

Determination of cyamemazine affinity for five subtypes of human serotoninergic receptors revealed that the compound had very high affinity for h5-HT$_{2A}$ receptors (Table 2, $K_i = 1.5 \pm 0.7$ nM). The affinity for the h5-HT$_{2A}$ receptor subtype (1.5 nM) was 4-fold higher than that for hD$_2$ receptors (5.8 nM). Of the remaining subtypes of human serotoninergic receptors, Fig. 2 and Table 2 show that the compound had affinity in the nanomolar range for h5-HT$_{2C}$ ($K_i = 11.8 \pm 2.2$ nM) and h5-HT$_7$ ($K_i = 22$ nM) receptors but only in the micromolar range for h5-HT$_{1A}$ ($K_i = 0.517$ μM) and h5-HT$_3$ ($K_i = 2.9 \pm 0.4$ μM) receptors all expressed CHO cells, except for the h5-HT$_3$ which was expressed in HEK-293 cells.

Interestingly, cyamemazine was 40-fold more potent in displacing the high affinity radioligand [^{3}H]BRL 43694 from 5-HT$_3$ receptors natively present in N1E-115 cells ($K_i = 75 \pm 9$ nM) than from the recombinant h5-HT$_3$ expressed in HEK-293 cells (Table 2).

3.3. Other receptors

3.3.1. Human recombinant muscarinic receptor subtypes

Cyamemazine exhibited a nanomolar affinity for the five subtypes of human muscarinic receptors hM$_1$ ($K_i = 13$ nM), hM$_2$ ($K_i = 42$ nM), hM$_3$ ($K_i = 32$ nM), hM$_4$ ($K_i = 12$ nM), and hM$_5$ ($K_i = 35$ nM) expressed in CHO cells (Table 2).

3.3.2. Non-selective rat cerebral cortex α_1- and α_2-adrenoceptors

Cyamemazine exhibited a very low affinity, in the micromolar range ($K_i = 1.3$ μM) for the non-selective α_2-adrenoceptor determined by using radiolabeled yohimbine in rat cerebral cortex membrane preparations (Table 2). However, cyamemazine had a high potency ($K_i = 2.3$ nM) in antagonizing radiolabeled prazosin binding to α_1-adrenoceptors present in rat cerebral cortex (Table 2).

3.3.3. Native animal brain GABA and histamine receptor subtypes

Cyamemazine up to the concentration of 30 μM lacked measurable affinity for the GABA$_A$ and GABA$_B$ receptors in rat cerebral membrane preparations. However, under the same experimental conditions, the reference agents, muscimol (an agonist of GABA$_A$ receptors) and baclofen (an antagonist of GABA$_B$ receptors), displaced the high affinity binding of specific ligand with nanomolar potency (Table 2).

Cyamemazine was a potent antagonist ($K_i = 9.3$ nM) of radiolabeled pyrilamine specific binding to central H$_1$ receptors natively occurring in the guinea-pig cerebellum. However, cyamemazine exhibited a substantially (20-fold) lower affinity toward the H$_2$ histamine receptors in the guinea-pig striatum ($K_i = 351$ nM) and an extremely low affinity for H$_3$ histamine receptors ($K_i = 22$ μM) determined by using (*R*)-α-Me-histamine which displaced the high affinity radiolabeled ligand from its binding sites in membranes prepared from the rat cerebral cortex (Table 2).

4. Discussion

The major findings of this paper are that, in addition to dopamine receptors, h5-HT$_{2C}$ and h5-HT$_{2A}$ receptors can also be involved in the therapeutical action of cyamemazine in psychotic patients. First, h5-HT$_{2C}$ receptors, for which cyamemazine displayed an affinity ($K_i = 12$ nM) even higher than that previously found in rat brain membranes ($K_i = 27$ nM, [3]). Such a high affinity for 5-HT$_{2C}$ receptors can explain, at least in part, its anxiolytic activity in humans (see later). Second, of all human recombinant receptor subtypes studied here, the highest affinity of cyamemazine was for the h5-HT$_{2A}$ subtype ($K_i = 1.5$ nM) and the affinity for this receptor was 4-fold higher than that for hD$_2$ receptors ($K_i = 5.8$ nM).

It may be objected that the affinity of a compound for a receptor demonstrated in a classical radioligand binding assay does not indicate whether it results from an agonist or an antagonist action. However, functional studies in animals indicate that cyamemazine behaves as an antagonist of 5-HT$_{2C}$ receptors [3]. Hence, it is reasonable to advance the hypothesis that the affinity of cyamemazine for h5-HT$_{2C}$ receptors is due to an antagonist activity and this effect can contribute to anxiolytic property of cyamemazine clearly observed in patients treated with this drug. Indeed, ritanserin possesses this receptor profile and is considered an effective anxiolytic agent in humans ([25]; see, however, [26]). Furthermore, recent pharmacological studies have further substantiated an anxiolytic potential for 5-HT$_{2C}$ receptor antagonists ([27–29]; see, however, [30]).

Cyamemazine exhibited low affinity for h5-HT$_3$ receptors ($K_i = 3$ μM), a finding contrasting with previous findings in rodent preparations, where cyamemazine bound at 5-HT$_3$ receptors [31] and antagonized 5-HT-induced contractions in guinea-pig ileum with a K_i value of 30 nM [3]. The later high affinity value for 5-HT$_3$ receptors was confirmed here in murine neuroblastoma N1E-115 cells

(K_i = 75 nM) which express this receptor subtype. Therefore, 5-HT_3 receptor can perhaps play a role in the anxiolytic activity of cyamemazine in mice [4]. However, it seems unlikely that this receptor is involved in cyamemazine-induced anxiolysis in humans [1,2]. An alternative explanation is that different 5-HT_3 receptor subtypes exist in the different preparations studied (for recent review of pharmacological heterogeneity and evidence of 5-HT_3 receptor subtypes see [32]).

The present results allow to exclude two additional potential receptor mechanisms for the anxiolytic activity of cyamemazine. These are $GABA_A$ and α_2-adrenergic receptors for which cyamemazine exhibited a very low affinity (>30 μM and 1320 nM, respectively). Finally, the *h*5-HT_{1A} affinity is weak (517 nM), but it can also contribute to the anxiolytic activity of cyamemazine. Whether serotonin reuptake, or the chloride channel coupled to $GABA_A$ receptors or other possible mechanisms mediate partly the anxiolytic properties of cyamemazine remains an issue for further investigation.

An unanticipated finding was the very high affinity of cyamemazine for *h*5-HT_{2A} receptors (K_i = 1.5 nM). As pointed out earlier, cyamemazine shares this property with the group of the so-called atypical antipsychotics [23,24] and this may explain its known low propensity to produce extrapyramidal adverse reactions in patients. Conversely, 5-HT_{2A} antagonism is unlikely to contribute to the anxiolytic activity of cyamemazine, since clozapine which shares the same property lacks anxiolytic effects in both isolated and group-housed male mice in the elevated plus-maze test [33]. Concerning *h*5-HT_{2A} receptors, it is important to mention that high affinity for cyamemazine was recently confirmed by Positron Emission Tomography (Yann Hode, personal communication).

Roth *et al*. have found that atypical neuroleptics bind with high affinity to cloned 5-HT_{2C} receptors (7–30 nM, see [34]) and to rat 5-HT_7 receptors (pimozide had the highest affinity = 0.5 nM, see [35]). Cyamemazine also showed high affinity for *h*5-HT_{2C} receptors (12 nM) and to *h*5-HT_7 receptors (22 nM).

Cyamemazine exhibited high affinity for dopamine D_2 receptors (6 nM) consistent with its antipsychotic action in schizophrenia. Similarly to clozapine, cyamemazine also exhibited high affinity for dopamine D_1 receptors (4 nM). There are a number of clinical and laboratory observations, consistent with the notion that the interaction of clozapine with D_1 receptors is involved in its beneficial role in schizophrenia, particularly concerning sedation [36–38]. Whether the interaction with dopamine D_1 receptors is involved in the therapeutical action of cyamemazine deserves further investigation.

Two other findings deserve a brief comment. First, cyamemazine affinity for H_1 histamine receptors can explain the sedative effects associated with the use of high doses of cyamemazine. Second, cyamemazine affinity for M_3 acetylcholine receptors may mediate the mouth dryness, experienced also by patients receiving high doses of this drug.

In conclusion, similarly to clozapine and other atypical antipsychotic agents, cyamemazine possesses high affinity for *h*5-HT_{2A}, *h*5-HT_{2C}, and *h*5-HT_7 receptors, a property which can explain its low propensity to produce extrapyramidal adverse reactions. Moreover, the high affinity of cyamemazine for *h*5-HT_{2C} receptors is likely to contribute to its anxiolytic action in humans.

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