ELSEVIER

European Journal of Pharmacology 454 (2002) 235–239

www.elsevier.com/locate/ejphar

5-HT_{2A} receptor antagonist properties of cyamemazine in rat and guinea pig smooth muscle

Miriam Alvarez-Guerra[a], Ahcène Hameg[b], Franck Bayle[c], Michel Dib[b], Ricardo P. Garay[a,*]

[a] *INSERM U400, Faculté de Médecine de Créteil, 8 rue du Général Sarrail, 94010 Créteil Cédex, France*
[b] *Aventis, Paris, France*
[c] *Inserm EMI-E0117, Université Paris V, 75014 Paris, France*

Received 16 September 2002; accepted 26 September 2002

Abstract

5-HT_{2A} receptor antagonism seems to explain the low incidence of extrapyramidal side effects with atypical neuroleptics. Whether the neuroleptic cyamemazine, which at low doses is also devoid of extrapyramidal side effects, possesses 5-HT_{2A} receptor antagonist properties is unknown. Cyamemazine was tested for its ability to antagonize 5-HT_{2A}-mediated responses in isolated rat aorta and guinea pig trachea and to displace [^{3}H]ketanserin specifically bound to rat brain membranes. In isolated rat aorta, cyamemazine potently and competitively antagonized serotonin-dependent contractions ($pA_2 = 8.82 \pm 0.26$, $n = 7$; Schild's slope $= 1.02 \pm 0.29$). In this test, cyamemazine was of similar potency as ketanserin ($pA_2 = 8.23$). In isolated guinea pig trachea, cyamemazine reduced maximum contractile responses to serotonin with $pIC_{50} = 7.92 \pm 0.35$, ($n = 4$), whereas ketanserin exhibited a $pIC_{50} = 8.79$. Finally, cyamemazine displaced [^{3}H]ketanserin specifically bound to rat brain membranes with $pK_i = 8.76 \pm 0.53$ ($n = 3$). In conclusion, cyamemazine behaves as a potent antagonist at 5-HT_{2A} receptors, which compares well with the reference compound, ketanserin. Whether this 5-HT_{2A} receptor antagonist action of cyamemazine can explain its low incidence of extrapyramidal side effects deserves further investigation.

Keywords: Cyamemazine; Serotonin; 5-HT_{2A} receptor; Neuroleptic; Atypical neuroleptic

1. Introduction

Cyamemazine is a neuroleptic dopamine D_2 receptor antagonist, which at low doses is devoid of extrapyramidal actions, but possesses anxiolytic properties in mice and humans (Paleologue and Varouchakis, 1976; Dubroca, 1989; Radat, 1995; Bourin et al., 2001). Anxious behaviour is modulated by central serotoninergic mechanisms in both animals and humans (for review see Leonard, 1996; Deakin, 1998) and we previously showed that cyamemazine possesses potent antagonistic properties at 5-HT_3 and 5-HT_{2C} receptor subtypes in guinea pig smooth muscle and rat brain membranes, respectively (Alvarez-Guerra et al., 2000).

Reduction of extrapyramidal actions by clozapine and other atypical neuroleptics has been explained by antagonistic actions at 5-HT_{2A} receptors (for review see Bhana et al., 2001; Meltzer, 1999; Alcantara and Barcia, 1999; Bymaster et al., 1999). A previous radioligand binding study showed that cyamemazine possesses affinity for 5-HT_{2A} receptors (Garay and d'Alché-Birée, 1995). Therefore, the present study was undertaken to establish whether cyamemazine antagonizes 5-HT_{2A}-mediated responses in isolated rat aorta and guinea pig trachea, and whether it compares with reference compounds. Cyamemazine was also tested for its ability to displace [^{3}H]ketanserin specifically bound to rat brain membranes.

2. Materials and methods

The investigation was performed according to the European Community guidelines for animal ethical care and the Guide for care and use of laboratory animals published by the US National Institute of Health (NIH publication No. 85-23, revised 1985).

* Corresponding author. Tel.: +33-1-4981-9424/25; fax: +33-1-6904-3251.
E-mail address: garayperso@aol.com (R.P. Garay).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02489-5

2.1. Serotonin-induced contraction of isolated rat aortic rings

Male Wistar rats weighing 250–300 g were deprived of food the evening and the morning before the experiment. Anesthesia was induced with pentobarbital sodium (60 mg/kg, i.p.). After cervical dislocation, their thoracic aortas were immediately removed and carefully cleaned. The endothelium was removed by gently rubbing the intimal surface of the aorta with a small wood stick. The thoracic aorta was cut into four rings (3 mm long) that were individually placed between platinum hooks in 25 ml Krebs solution maintained at 37 °C and gassed with 95% O_2/5% CO_2. The Krebs medium contained (mM): NaCl 118, $NaHCO_3$ 25, $CaCl_2$ 1.25, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.5. An initial load of 1 g was applied to the preparations and maintained throughout a 90-min equilibration period during which the incubation medium was renewed every 20 min. Tension was recorded on a MacLab (Analog Digital Instruments, Castle Hill, Australia) via Dynamometers Pioden Control UF1 (distributed through PHYMEP, Paris, France). Then, cumulative concentration–tension response curves to serotonin were obtained in aortic rings incubated 20 min before, and during the building up of the concentration–response to the vasoconstrictor, with cyamemazine (final concentration: 0.1, 0.3, 1, 3, 10 and 30 nM), ketanserin (0.3, 1, 3, 10 and 30 nM), or their vehicle. An absence of functional endothelium was indicated by the failure of the preparation precontracted with a submaximum concentration of phenylephrine to relax to acethylcholine (1 μM). Basal contraction values were 1005 ± 4 mg ($n = 45$). Serotonin (100 μM) contracted rat aorta up to a maximum value of 2908 ± 228 mg ($p < 0.05$, non-paired Student's *t*-test).

2.2. Serotonin-induced contraction of isolated guinea-pig trachea

Hartley guinea pigs (250 g) were anesthetized with sodium pentobarbital and the trachea was isolated and carefully cleaned in a Petri dish containing warm Krebs solution (see below for composition) and cut in segments. Then, the segments were individually placed between platinum hooks in 25 ml chambers with Krebs solution (37 °C) containing in mM: 118 NaCl, 2.8 KCl, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11 glucose and continuously gassed with 95% O_2/5% CO_2. Then, cumulative tension response curves to serotonin were obtained (maximum responses, with serotonin 10 μM, ranged from 600 to 1200 mg) and compounds were tested for 5-HT_{2A} receptor antagonist properties as described above for rat aorta.

2.3. Binding studies

Binding of [^{3}H]ketanserin to rat cortical membranes was investigated according to the method of Leysen et al. (1982). Briefly, membranes were incubated for 15 min at 37 °C in 50 mM Tris–HCl (pH 7.6) containing 1 nM of [^{3}H]ketanserin (60–90 Ci/mmol) with and without cold methysergide (100 μM) to estimate non-specific binding. The reaction was rapidly terminated by rapid vacuum filtration onto glass fiber filters and radioactivity trapped into the filters was determined. Specific binding always represented 65–80% of total binding. Maximal receptor number (B_{max}) was 31 fmol/mg humid tissue.

2.4. Compounds

Cyamemazine base was obtained from Rhône-Poulenc-Rorer (Vitry, France). Other compounds were from Research Biochemicals International (Natick, MA), Fisher (Itasca, IL) or Sigma (St. Louis, MO) and were of the highest grade possible. Concentrated solutions of cyamemazine and ketanserin in dimethylsulfoxide (DMSO) were prepared the day of the experiment and diluted in incubation media, provided that the final DMSO concentration had no effect on tissue contractility.

2.5. Statistical analysis

Values are given as mean ± S.E.M. Concentration–response curves to serotonin were fitted by linear regression analysis of the straight portions of the contraction responses. From the results of this analysis, the concentration of serotonin given 50% of the maximal response was calculated ($ED_{50\%}$). Relative antagonistic potencies (pA_2: − log of the concentration of an antagonist causing a twofold, rightwards displacement of the control serotonin–concentration response curve) were estimated by applying the Schild analysis (Arunlakshana and Schild, 1997).

3. Results

3.1. Effects of cyamemazine on serotonin-dependent contraction in isolated rat aorta

Cyamemazine was tested in isolated rat aorta for its agonist and antagonist contractile effects at 5-HT_{2A} serotonin receptors. Cyamemazine was unable to contract isolated rat aorta per se. Thus, tension values in the presence of the maximal cyamemazine concentration tested (30 nM) were not significantly different from basal values (1013 ± 14 vs. 996 ± 10 mg for cyamemazine and vehicle, respectively, $n = 3$, non-significant, non-paired Student's *t*-test).

Fig. 1 (upper panel) shows contractile responses to cumulative concentrations of serotonin, under control conditions and in the presence of several concentrations of cyamemazine. It can be seen that cyamemazine produced a concentration-dependent rightward shift of the control contractile responses to serotonin. Schild plot of the data (Fig. 1,

lower panel) allowed to calculate a $pA_2 = 8.82 \pm 0.26$ ($n = 7$) for cyamemazine (slope of Schild plot was 1.02 ± 0.29).

Ketanserin, a potent 5-HT_{2A} receptor antagonist, was tested under similar conditions as cyamemazine. Fig. 1 (lower panel) shows Schild plot of the data, which allowed to calculate a $pA_2 = 8.23 \pm 0.25$ ($n = 4$) for ketanserin (slope of Schild plot was 0.88 ± 0.15).

3.2. Effects of cyamemazine on serotonin-dependent contraction in isolated guinea pig trachea

Cyamemazine was tested in isolated guinea pig trachea for its antagonist contractile effects at 5-HT_{2A} serotonin receptors. Fig. 2 (upper panel) shows contractile responses to cumulative concentrations of serotonin, under control conditions and in the presence of several concentrations of cyamemazine. It can be seen that cyamemazine produced a concentration-dependent decrease of the maximum contractile responses to serotonin. This is illustrated in Fig. 2 (lower panel) where for each serotonin concentration, the % contraction was plotted as a function of cyamemazine concentration. It can be seen that all curves superposed, as expected for non-competitive inhibition of maximum responses. Calculated cyamemazine pIC_{50} values were 7.92 ± 0.35 ($n = 4$).

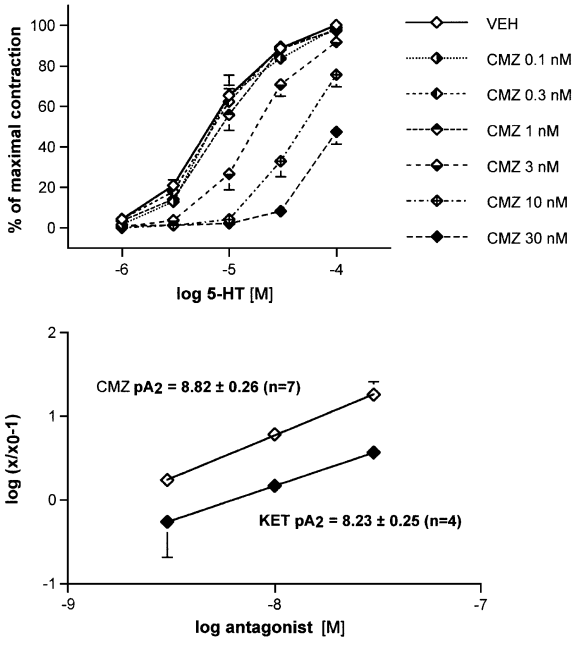

Fig. 1. Antagonism by cyamemazine (CMZ) of serotonin-dependent contraction of isolated rat aorta. Upper panel: Concentration–response curves for serotonin (5-HT) expressed as percentage of the maximal control tension responses in isolated rat aorta, under normal conditions and in the presence of several concentrations of cyamemazine (CMZ). Values are given as mean ± S.E.M. ($n = 7$). It can be seen that cyamemazine produced a parallel concentration-dependent rightwards shift of the control contractile responses to serotonin. Lower panel: Schild plot of the data. Values are given as mean ± S.E.M. Slope of Schild plot was 1.02, suggesting that cyamemazine displays competitive kinetics. Results obtained with a reference compound (ketanserin = KET) are given for comparison.

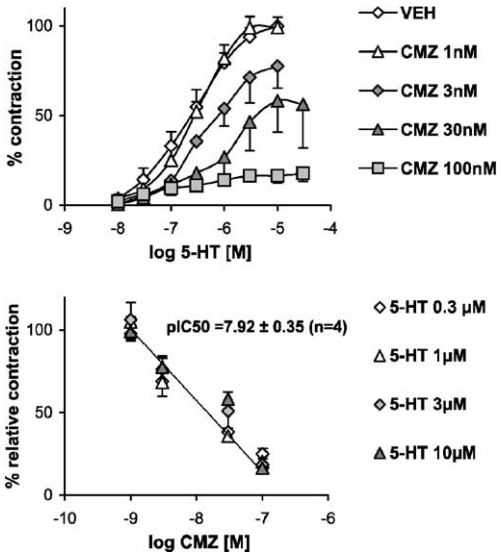

Fig. 2. Antagonism by cyamemazine (CMZ) of serotonin-dependent contraction of isolated guinea pig trachea. Upper panel: Concentration–response curves for serotonin (5-HT) expressed as percentage of the maximal control tension responses in isolated guinea pig trachea, under normal conditions and in the presence of several concentrations of cyamemazine (CMZ). Values are given as mean ± S.E.M. ($n = 4$). It can be seen that cyamemazine produced concentration-dependent reduction of maximum contractile responses to serotonin. Lower panel: % of relative contraction as a function of log cyamemazine concentration at different, constant serotonin concentrations. All curves superposed, as expected for non-competitive inhibition of maximum responses. Values are given as mean ± S.E.M.

Ketanserin, a potent 5-HT_{2A} receptor antagonist, was tested under similar conditions as cyamemazine. Similarly to cyamemazine, ketanserin produced a concentration-dependent decrease of the maximum contractile responses to serotonin, with $pIC_{50} = 8.79 \pm 0.15$ ($n = 4$).

3.3. Binding to 5-HT_{2A} receptors

[^{3}H]Ketanserin specifically bound to rat brain membranes was displaced by cyamemazine with $pK_i = 8.76 \pm 0.53$ ($n = 3$). Affinity of cyamemazine for serotonin 5-HT_{2A} receptors ($pK_i = 8.76$) was as high as that of the comparative molecules:ketanserin ($pK_i = 9.4$) and methysergide ($pK_i = 8.89$).

4. Discussion

Cyamemazine acted as a potent serotonin 5-HT_{2A} receptor antagonist in both, isolated rat aorta and isolated guinea pig trachea. The 5-HT_{2A} receptor antagonist potency of cyamemazine was: (i) comparable to that of the reference compound (ketanserin) and (ii) higher than that previously observed (Alvarez-Guerra et al., 2000) to antagonize 5-HT_3 or 5-HT_{2C} receptor-evoked responses in the same animal species. These results can explain the low incidence of extrapyramidal side actions by cyamemazine (for 5-HT_{2A}

receptors and extrapyramidal side actions, see Bhana et al., 2001; Meltzer, 1999; Alcantara and Barcia, 1999; Bymaster et al., 1999).

In isolated rat aorta and guinea pig trachea, serotonin evokes important contractile responses mediated by stimulation of 5-HT_{2A} receptors (Baez et al., 1994). In both preparations, cyamemazine antagonized serotonin-dependent contractions in the low nanomolar range. However, whereas in rat aorta cyamemazine behaved like a competitive antagonist (Schild plot's slope not different from 1), a reduction of maximum contraction was seen in guinea pig trachea (Fig. 2, top).

In guinea pig trachea, cyamemazine IC_{50} was independent of serotonin concentration (Fig. 2, bottom) for serotonin concentrations ≥ 0.3 μM (serotonin concentrations which produce tracheal contractions $\geq 55\%$ of maximum, see vehicle curve in Fig. 2A). Thus, cyamemazine behaved as a non-competitive antagonist, at least in the 55–100% range of maximal serotonin-induced tracheal contractions (the experimental precision does not allow to exclude competitive antagonism for smaller contractions, see Fig. 2, top). If non-competitive antagonism is taken for granted, cyamemazine IC_{50} (12 nM) can then be equated to the K_i.

The 5-HT_{2A} receptor antagonist potency of cyamemazine in rat aorta ($pA_2 = 8.82$) compared well with the affinity for 5-HT_{2A} receptors in rat brain membranes ($pK_i = 8.76$). Moreover, 5-HT_{2A} receptor antagonist potency in rat aorta ($K_i = 1.5$ nM) was 8 times higher as in guinea pig trachea ($K_i = 12$ nM).

The quali- and quantitative differences in 5-HT_{2A} receptor antagonist properties of cyamemazine between rat aorta and guinea pig trachea can be explained by the unusual molecular and functional properties of the 5-HT_{2A} receptor in the guinea pig. Thus, Watts et al. (1994a) have found that the guinea pig 5-HT_{2A} receptor has two tandem substitutions that disrupted a potential alpha helix in the region of the third cytoplasmic loop, which seems involved in the intracellular coupling of the receptor. Thus, the 5-HT_{2A} receptor in guinea pig trachea is unusual in that activation of the receptor does not result in phosphoinositide hydrolysis but increases calcium influx through L-type voltage dependent calcium channels, calcium release from the sarcoplasmic reticulum and activation of protein kinase C (Watts et al., 1994b). Moreover: (i) in guinea pig trachea, serotonin contractile responses are much higher as in rat trachea (Baez et al., 1994) and (ii) guinea pig aorta contracted less dramatically to serotonin than rat aorta, in spite of a similar concentration of 5-HT_{2A} receptor specific mRNA in both preparations (Baez et al., 1994).

The 5-HT_{2A} receptor antagonist potency of cyamemazine ($K_i = 1.5$ and 12 nM in rat aorta and guinea pig trachea, respectively): (i) compares well with the 5-HT_{2A} receptor antagonist potency of the reference compound ketanserin ($K_i = 5.9$ and 1.6 nM in rat aorta and guinea pig trachea, respectively), (ii) is 280 times higher as compared with its 5-HT_{2C}-antagonistic potency in rat brain membranes ($K_i = 424$ nM; Alvarez-Guerra et al., 2000) and is 3 times higher than its 5-HT_3-antagonistic potency in guinea pig ileum ($K_i = 30$ nM, Alvarez-Guerra et al., 2000). Therefore, the high 5-HT_{2A} receptor antagonist potency of cyamemazine can perhaps explain its therapeutical profile.

One main property explaining the reduction of extrapyramidal actions with atypical neuroleptics seems to be an antagonistic action at 5-HT_{2A} receptors (for review see Bhana et al., 2001; Meltzer, 1999; Alcantara and Barcia, 1999; Bymaster et al., 1999). In this respect, cyamemazine compares well ($K_i = 1.5–12$ nM) with clozapine ($K_i = 6.5$ nM), olanzapine ($K_i = 2.5$ nM, from Bymaster et al., 1999) and risperidone ($K_i = 0.12$ nM, from Alcantara and Barcia, 1999). Thus, serotonin 5-HT_{2A} receptor antagonism may explain the low incidence of extrapyramidal side effects observed with cyamemazine. Such an hypothesis deserves further investigation. Finally, whether serotonin 5-HT_{2A} receptor antagonism can participate to the anxiolytic properties of cyamemazine also remains as an open question.

In conclusion, cyamemazine behaves as a potent antagonist at 5-HT_{2A} receptors, which compares well with reference compounds. This 5-HT_{2A} receptor antagonist action of cyamemazine can participate in its therapeutical profile, particularly the low incidence of extrapyramidal side effects.

Acknowledgements

We are greatly indebted to E. Garay (CRAVEN, Paris, France), who helped with the coordination and monitoring of this study.

References

Alcantara, A.G., Barcia, D., 1999. Risperidone and concept of bipolar neuroleptic. Encephale 25, 146–150.

Alvarez-Guerra, M., d'Alché-Birée, F., Wolf, W.A., Vargas, F., Dib, M., Garay, R.P., 2000. 5-HT_3- and 5-HT_{2C}-receptor antagonist properties of cyamemazine: significance for its clinical anxiolytic activity. Psychopharmacology 147, 412–417.

Arunlakshana, O., Schild, H.O., 1997. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 120 (4 Suppl.), 151–161 (discussion 148-50).

Baez, M., Mercurio, L., Schenck, K., Cohen, M.L., 1994. Relationship between 5-HT2A receptor mRNA density and contractility in trachea and aorta from guinea pig and rat. Life Sci. 55, 105–114.

Bhana, N., Foster, R.H., Olney, R., Plosker, G.L., 2001. Olanzapine: an updated review of its use in the management of schizophrenia. Drugs 61, 111–161.

Bourin, M., Dhonnchadha, B.A.N., Colombel, M.C., Dib, M., Hascoët, M., 2001. Cyamemazine as an anxiolytic drug on the elevated plus maze and light/dark paradigm in mice. Behav. Brain Res. 124, 87–95.

Bymaster, F., Perry, K.W., Nelson, D.L., Wong, D.T., Rasmussen, K., Moore, N.A., Calligaro, D.O., 1999. Olanzapine: a basic science update. Br. J. Psychiatry 37, 36–40 (Supplement).

Deakin, J.F., 1998. The role of serotonin in panic, anxiety and depression. Int. Clin. Psychopharmacol. 13 (Suppl. 4), S1–S5.

Dubroca, J.M., 1989. L'anxiolyse: intérêt de la cyamémazine. Neuro-psy, 4–7.

Garay, R.P., d'Alché-Birée, F., 1995. Cyamémazine (Tercian^{R}): profil neuroleptique ou anxiolytique? Inf. Psychiatr. 10, 969–970.

Leonard, B.E., 1996. Serotonin receptors and their function in sleep, anxiety disorders and depression. Psychoter. Psychosom. 65, 66–75.

Leysen, J.E., Niemegeers, C.J., Van Nueten, J.M., Laduron, P.M., 1982. [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin2 receptor binding sites. Binding properties, brain distribution, and functional role. Mol. Pharmacol. 21, 301–314.

Meltzer, H.Y., 1999. The role of serotonin in antipsychotic drug action. Neuropsychopharmacology 21 (Suppl. 2), 106S–115S.

Paleologue, A., Varouchakis, H., 1976. Etude de l'activité d'un nouveau neuroleptique. Inf. Psychiatr. 52, 249–256.

Radat, F., 1995. Cyamémazine: traitement symptomatique des dimensions anxieuses, impulsives et agressives. Inf. Psychiatr. 10, 967–968.

Watts, S.W., Cohen, M.L., Mooney, P.Q., Johnson, B.G., Schoepp, D.D., Baez, M., 1994a. Disruption of potential alpha-helix in the G loop of the guinea pig 5-hydroxytryptamine2 receptor does not prevent receptor coupling to phosphoinositide hydrolysis. J. Neurochem. 62, 934–943.

Watts, S.W., Cox, D.A., Johnson, B.G., Schoepp, D.D., Cohen, M.L., 1994b. Contractile serotonin-2A receptor signal transduction in guinea pig trachea: importance of protein kinase C and extracellular and intracellular calcium but not phosphoinositide hydrolysis. J. Pharmacol. Exp. Ther. 271, 832–844.