Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3779–3782

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

The Acute EPS of Haloperidol May Be Unrelated to Its Metabolic Transformation to BCPP$^+$

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Received 14 May 2003; accepted 30 July 2003

Abstract—We have previously proposed that haloperidol's debilitating extrapyramidal symptoms (EPS) may be associated with its quaternary BCPP$^+$ (an MPP$^+$ like species) metabolite formed in vivo. However, recent work on D2 knock out mice suggests that haloperidol's EPS may be related to its potent D2 binding ($K_i = 0.9$ nM). In this study, we explore this question by synthesizing and testing an analogue (DS-27) that binds to D2 receptors with higher affinity than haloperidol, but cannot form quaternary metabolites. This study suggests that D2 affinity may be the primary underlying mechanism for acute catalepsy induction by haloperidol.

Haloperidol (**1**) is a drug of choice in the treatment of schizophrenia and acts in part by inhibiting dopamine receptors in the CNS.[1] Unfortunately, haloperidol also precipitates debilitating movement disorders in the form of acute EPS and chronic irreversible tardive dyskinesia or TD.[2–4] The mechanism by which haloperidol induces extrapyramidal symptoms (EPS) is still a subject of ongoing research. It has been shown that haloperidol is oxidatively biotransformed to a neurotoxic metabolite BCPP$^+$ (HPP$^+$), and that BCPP$^+$ destroys dopamine neurons.[5–7] Persistent reports, however, point to high D2 binding by haloperidol in the brain's nigrostriatal areas as the cause for acute EPS.[8–10] Therefore, we have developed a working hypothesis stating that the acute EPS associated with haloperidol is related to its potent D2 binding affinity and not to the formation of BCPP$^+$.[11,12]

If BCPP$^+$ contributes to the acute EPS associated with haloperidol, then an analogue such as of 4-[5-*trans*-hydroxy-5-*cis*-(4′-chlorophenyl)-2-azabicyclo[3.2.1]octanyl]-4″-fluorobutyrophenone, DS27 (**2**) which could not undergo biotransformation to BCPP$^+$-like species should be devoid of catalepsy. On the other hand, if analogue **2** produces catalepsy during the acute phase, then BCPP$^+$ is not a major contributor to catalepsy induction in rats.

To test our hypothesis, we synthesized **2**, an analogue that is incapable of forming BCPP^{+-} like metabolites (via dehydration and oxidation) since a double bond could not be formed at the bridgehead of the 8-azabicyclo [3.2.1] octane in violation of Bredt's rule.[13] Compound **2** however, binds to D2 receptors with 3-fold higher affinity ($K_i = 0.31$ nM) than haloperidol ($K_i = 0.89$ nM). We then evaluated **2** for its ability to antagonize dopamine binding to its receptors using the apomorphine induced climbing test in male Swiss mice, and for its cataleptogenic effects in male Sprague–Dawley (SD) rats in a 'bar test.'[14] Catalepsy induction in animals is an indication of a drug's propensity to induce EPS in humans. Haloperidol and Clozapine (**4**) were

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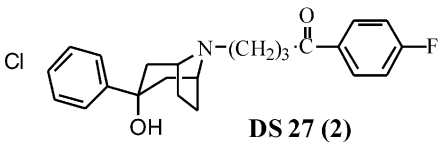
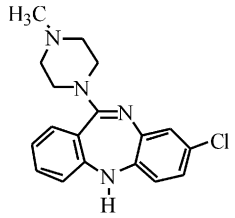
doi:10.1016/j.bmcl.2003.07.015



DS 27 (2)



Clozapine (3)

used as respective positive and negative controls in both tests.

Scheme 1 depicts the synthesis of **2**. A Grignard reaction involving 4-chlorophenyl magnesium bromide and *N*-carbethoxy-4-tropinone **4** produced the tertiary alcohol **5** in a stereoselective manner to yield the *endo* alcohol, **5**. Heating **5** and KOH in ethylene glycol yielded a decarbamylated free secondary amine **6**. The free amine was then alkylated with 4-iodo-4′-fluorobutyrophenone to give **2**.

Binding Affinities

Haloperidol (**1**) and **2** were evaluated in vitro for binding to human D2-like (D2, D3 and D4) receptors. Radioligand binding studies were performed according to standard procedures.[15] Briefly, appropriate weight of frozen cell paste expressing human dopamine D2, D3 or D4 receptors was homogenized using a Brinkman Polytron model PT3000 (setting 15,000 rpm, 15 s) in 50 mM Tris–HCl buffer pH 7.4 containing 2 mM $MgCl_2$. The homogenate was centrifuged for 10 min at 40,000*g*, washed and recentrifuged. The final pellet was resuspended in 50 mM Tris–HCl buffer pH 7.4 containing 100 mM NaCl and 1 mM $MgCl_2$ for the D2 cell homogenate; 50 mM Tris–HCl buffer pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 5 mM $MgCl_2$ for the D3 cell homogenate, and 50 mM Tris–HCl buffer pH 7.4 containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ for the D4 cell homogenate. Incubations were initiated by the addition of tissue homogenate to 96-well plates containing ^{3}H-spiperone (0.20 nM, 0.10 nM, for D2 and D4 assays, respectively) or ^{3}H-7OH-DPAT (0.40 nM, for D3 assays) and varying concentrations of test compound, buffer or (+)-butaclamol in a final volume of 250 μL. Nonspecific binding was defined as the radioactivity remaining in the presence of a saturating concentration of a known inhibitor [10 μM (+)-butaclamol]. After a 15-min incubation at 37 °C for D2 and D3 receptor assays or a 45-min incubation at 30 °C for D4 receptor assays, assay samples were filtered onto GF/B filtermats that had been presoaked in 0.5% polyethylenimine, using a Skatron cell harvester (Molecular Devices) and washed with ice-cold 50 mM Tris buffer pH 7.4. Radioactivity was quantified by liquid scintillation counting (Betaplate, Wallac Instruments). The IC_{50} value (concentration at which 50% inhibition of specific binding occurs) was calculated by linear regression of the concentration–response data. K_i values were calculated according to Cheng and Prusoff, where $K_i = IC_{50}/[1 + (L/K_d)]$, where L is the concentration of the radioligand used in the experiment and the K_d value is the dissociation constant for the radioligand (determined previously by saturation analysis). The data is summarized in Table 1.

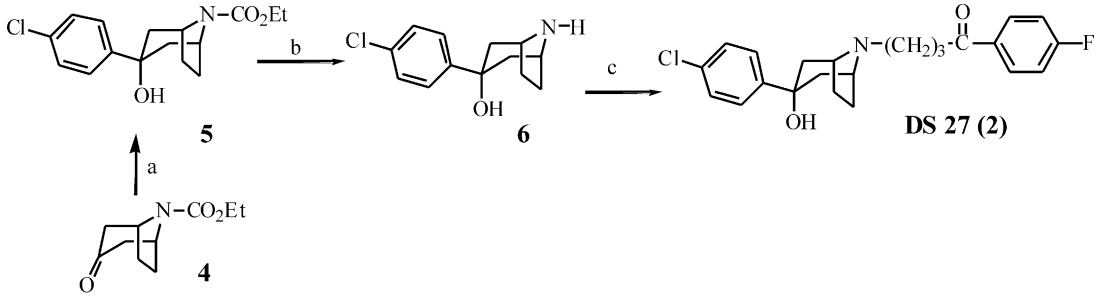
Apomorphine induced climbing-stereotypy

A modified climbing test by Needham et al.[14] was used. Swiss male mice (20–25 gm, $N = 125$) in groups of 5 per time point (30 min, 1, 2, 4, and 6 h) were injected ip with 0.1 mL/kg of vehicle (0.1% lactic acid) or increasing mol/kg equivalent doses of dopamine antagonists **1**, **2** (i.e., 1.9×10^{-6}; 5.3×10^{-6}), and **3** (9.2×10^{-5}; 2.4×10^{-4}). Animals were then challenged with apomorphine (0.88

Table 1. In vitro binding affinities [K_i (nM) and pK_i±SEM (*n*)] for cloned human DA receptors (D2, D3, and D4) by haloperidol, clozapine and compound **2**

Compd	K_i (nM) **D2** pK_i	K_i (nM) **D3** pK_i	K_i (nM) **D4** pK_i
Haloperidol (**1**)	0.89	4.6	10
	9.05±0.30 (3)	8.34±0.27 (3)	7.98±0.28 (3)
DS-27 (**2**)	0.31	0.71	12
	9.51±0.05 (3)	9.15±0.23 (3)	7.93±0.10 (3)
Clozapine (**3**)[a]	130	240	54
	6.87±0.10 (3)	6.62 0.05 (10)	7.27±0.06 (36)

[a]See ref 13.



Scheme 1. Reagents and conditions: (a) 4-chlorophenyl magnesium bromide, diethyl ether (dry), N_2, reflux, 12 h, 47%; (b) KOH, ethylene glycol, N_2, 150, 4 h, 73–90%; (c) 4-iodo-4′-fluorobutyrophenone, K_2CO_3, DME (dry), N_2, reflux, 12 h, 23%.

mg/kg body weight), placed in cylindrical wire cages (12 cm in diameter, 14 cm in height), and observed for climbing behavior at 10 and 20 min post dose. Climbing behavior was assessed as follows: four paws on the cage floor = 0 score; two or three paws on the cage = 1 score; four paws on the cage = 2 scores. Scores were expressed as mean % climbing inhibition, and plotted in Figure 1.

Bar test for catalepsy

A modified bar test by Needham et al. was used.[14] Male SD rats (200–300 gm, $N = 100$) were injected subcutaneously with 1 mL/kg of vehicle (<0.005% acetic acid in H_2O) or increasing mol/kg equivalent doses of **1**, **2** (i.e., 1.9×10^{-6}; 5.3×10^{-6}), and **3** (9.2×10^{-5}, 2.4×10^{-4}). Catalepsy severity was assessed immediately at various time points (15, 30, 45, 60, and 90 min) post injection, by scoring how long the rat maintained both forepaws motionless on a horizontal metal bar (1.1 cm in diameter, 10 cm above the bench top in a box). A score of 1 was given for every 5 s (2 min maximum) the animal remained on the bar. Mean scores from five animals per time point were recorded for catalepsy (Fig. 2).

Statistical analysis

The Student *t*-test was used to compare the three compounds used in the animal behavioral tests. Results were considered significant at $p < 0.05$.

Table 1 lists binding affinities of the two analogues and the controls at D2-like receptors. Affinities were determined by competitive radioligand displacement assays. In order to assess their EPS potential (expressed as catalepsy), we were interested in the D2 subtype affinities

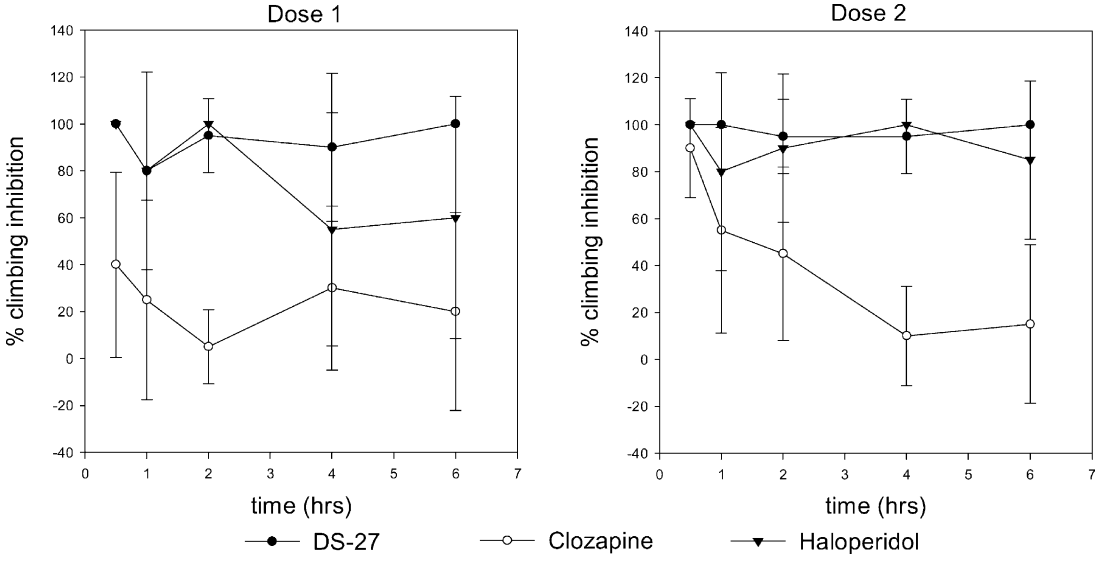


Figure 1. Percent inhibition of apomorphine induced climbing behavior at selected time points. Data represents mean values (±SEM) for $n = 10$ mice.

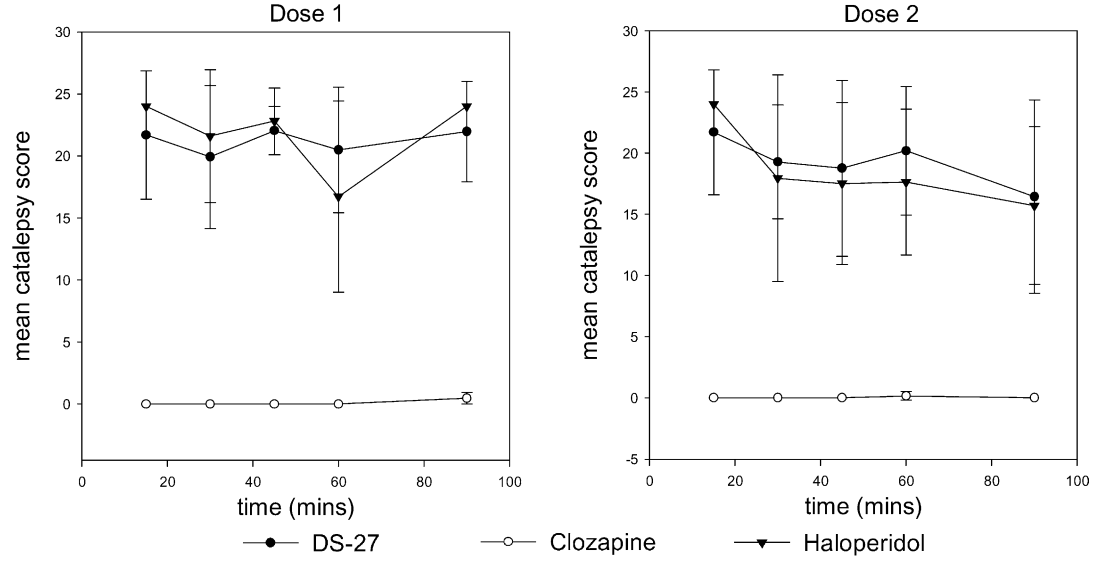


Figure 2. Mean catalepsy scores at different time points over a 90-min period. Data represents mean values (±SEM) for $n =$ five rats.

of the analogues since this appears to be associated with catalepsy.[15] Compound **2** exhibited a 3-fold higher affinity for D2 in comparison to haloperidol. Statistical analysis of both behavioral test data, revealed that compound **2**'s effects were significantly different from compound **3** but not significantly different from compound **1** at both doses (Figs. 1 and 2).

From this study, compounds **1**, **2** and **3** exhibited different binding affinities ($K_i = 0.89$, 0.31, and 130 nM) for the D2 subtype, respectively. Compound **2** showed a 3-fold higher binding affinity for D2 than haloperidol and a 400-fold higher affinity than **3**. Because **2** could inhibit apormophine induced climbing in mice, it meant that (like **1** and **3**) this compound was acting as a dopamine receptor antagonist. Further more, **2** exhibited catalepsy and inhibited apomorphine induced climbing in a statistically similar manner to **1**. Since compound **2** binds to D2 with high affinity and cannot form quaternary pyridinium species ($BCPP^+$), its cataleptogenicity cannot therefore be attributed to formation of quaternary pyridinium species but more likely to its D2 affinity. This data provides one more evidence in support of the hypothesis that haloperidol's high D2 binding may play a more central role in its acute EPS.

Acknowledgements

This study was supported by MBRS grant # GM 08111, RCMI grant # G12 RR 03020, Pfizer grant in support of MLE and Title III grants. We are grateful to Dr. Tanise Jackson, Dr. Nazarius Lamango, and Dr. Marshal Medou for their assistance with the animal work. We also gratefully acknowledge Drs Anne W. Schmidt, Michelle Vanase-Frawley, and Alka Shrihkande, for the binding data.

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