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Article

Receptor profile of P88-8991 and P95-12113, metabolites of the novel antipsychotic iloperidone

Natarajan Subramanian, Hans O. Kalkman*

Novartis Pharma AG, Research Nervous System, Basel, Switzerland

Abstract

Iloperidone is a novel atypical antipsychotic compound currently under clinical development for the treatment of psychotic disorders. In radioligand binding studies, iloperidone binds with high affinity to serotonin (5-HT) 5-HT_{2A} and noradrenaline α_1 and α_{2C} receptors [Neuropsychopharmacology (2001) 25, 904–914]. The human metabolism of iloperidone generates two major metabolites, P88-8991 and P95-12113. The aim of this study was to compare the receptor affinity profile of P88-8991 and P95-12113 with that of the parent compound. The receptor affinity profile of P88-8991 is comparable to that of iloperidone. This metabolite binds to the following monoamine receptors (pK_i values in nM): serotonin 5-HT_{2A} receptors (9.56), adrenergic α_1 (8.08) and α_{2C} (7.79) receptors, and D_{2A} receptors (7.80). Lower affinity is seen for other dopamine, serotonin, α_2-adrenergic and histamine H_1 receptors. In contrast, P95-12113 shows affinity for 5-HT_{2A} receptors (pK_i 8.15; which is 60-fold lower than that of iloperidone), adrenergic α_1 (7.67), α_{2C} (7.32) and α_{2B} (7.08) receptors. Given this affinity profile, and the observation that P95-12113 does not readily cross the blood–brain barrier, it is unlikely that this metabolite contributes to the therapeutic effect of iloperidone in patients with schizophrenia. However, the comparable receptor binding profile of P88-8991 indicates that it is likely to contribute to the clinical profile of iloperidone.

Keywords: Antipsychotic; Iloperidone; Metabolites; Pharmacology; Receptor

1. Introduction

The classical dopaminergic theory of schizophrenia ascribed the disease entirely to dopaminergic hyperactivity. Over the years, this view has been refined; positive symptoms are now thought to be due to overactivity in the mesolimbic dopaminergic pathways, whereas negative and cognitive symptoms are attributed to a reduction in activity in the mesocortical dopaminergic pathways. Thus, an antipsychotic agent should provide selective inhibition of dopaminergic function in the mesolimbic, but not in the cortical, brain regions (Deutch et al., 1991). One way to achieve selectivity between mesolimbic and mesocortical dopamine pathways is by concurrent serotonin (5-HT) 5-HT_2 and noradrenaline α_1 and α_2 receptor blockade (Svensson et al., 1995; Hertel et al., 1999). Clozapine inhibits dopamine, noradrenaline and 5-HT receptors (Schotte et al., 1996) and was the first atypical antipsychotic with clinical effectiveness against both positive and negative symptoms, without the potential for inducing the extrapyramidal symptoms (EPS) seen with classical antipsychotics (Kane et al., 1988). The low EPS liability and negligible effect on plasma prolactin levels of clozapine (Meltzer et al., 1989) are thought to be due to the drug having a much weaker D_2 receptor antagonism than classical antipsychotics (Kapur and Seeman, 2001). It is less clear why clozapine has a higher clinical response rate compared to that of the classical antipsychotics (Kane et al., 1988; Conley et al., 1999), but it is presumably due to concomitant blockade of additional neurotransmitter receptors. For example, antagonism at the adrenergic α_2 receptor has been reported to increase therapeutic responses in patients with schizophrenia (Litman et al., 1996).

Abbreviations: A, adenosine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CHO, Chinese hamster ovary; CCK, cholecystokinin; $CRF_{2\alpha}$, corticotropin-releasing factor 2α; EPS, extrapyramidal symptoms; GABA, γ-aminobutyric acid; $t_{1/2}$, half-life; MDCK, Madin–Darby canine kidney; NMDA, *N*-methyl-aspartate; M, muscarinic; NK, neurokinin; PAI, plasminogen activator inhibitor; 5-HT, serotonin; Sf9, spodoptera frugiperda

* Corresponding author. Lichtstrasse 35, WSJ-360.405, Basel CH-4002, Switzerland. Tel.: +41-61-324-4304; fax: +41-61-324-5474.

E-mail address: hans.kalkman@pharma.novartis.com (H.O. Kalkman).

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Iloperidone, a piperidinyl-benzisoxazole derivative, is a new atypical antipsychotic currently undergoing Phase III trials for the treatment of psychotic disorders. It was selected for development on the basis of its high affinity for rat 5-HT_2 receptors and moderate affinity for rat D_2 receptors in vitro (Corbett et al., 1997). In radioligand binding studies, iloperidone binds with high affinity to serotonin 5-HT_{2A}, dopamine D_3 and adrenergic α_1 receptors (Kongsamut et al., 1996; Kalkman et al., 2001). In addition, iloperidone has moderate to low affinity for D_1, D_2, α_{2A}, H_1 and muscarinic (M) receptors, which might be responsible for the reported good tolerability profile of the drug (Borison et al., 1996; Davidson et al., 1994).

P88-8991 and P95-12113 are the two main circulating human metabolites of iloperidone (Fig. 1). The aim of this study was to compare the receptor affinity profiles of iloperidone, P88-8991 and P95-12113, at a wide variety of neurotransmitter receptors. By comparing the profiles of the metabolites with the parent compound, the potential contribution of the metabolites to the therapeutic response following iloperidone administration may be better understood.

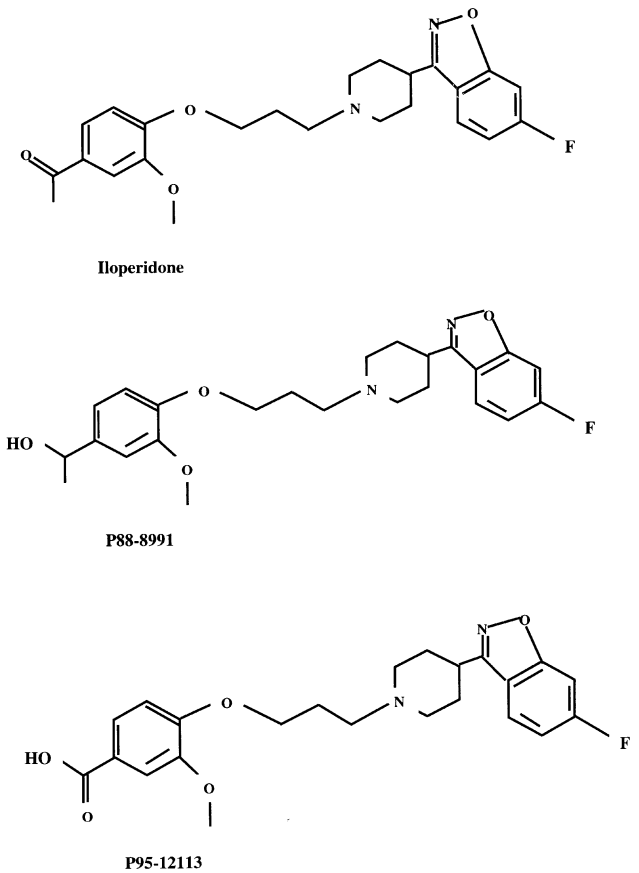

Fig. 1. The chemical structure of iloperidone and its two human metabolites, P88-8991 and P95-12113.

2. Methods

The radioligand receptor binding assays are listed in Table 1, and methodological details of receptor assays to which iloperidone and its metabolites had affinity are listed in Table 2.

2.1. Materials

Iloperidone and its metabolites were synthesized at Novartis Pharma (Basel, Switzerland). Radioligands were purchased from Perkin Elmer NEN Life Science Products, Boston, USA except, for ^{3}H RX821002 and ^{3}H Mesulergine, which were obtained from Amersham Pharmacia Biotech, Oxford, UK. Unless specified otherwise, all other chemicals were of reagent grade and obtained through standard commercial sources.

2.2. Cell lines and membranes

HEK293 cells expressing human recombinant D_{2A} receptors were obtained through Novartis Pharma. Membranes from Chinese hamster ovary (CHO) or CHO-K1 cells expressing human recombinant 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} receptors were obtained from EuroScreen, Brussels, Belgium. The following membrane preparations were purchased from Perkin Elmer NEN Life Science Products: NIH3T3 cells expressing human recombinant cholecystokinin (CCK) CCK_A and CCK_B; spodoptera frugiperda (Sf9) cells expressing recombinant human D_1, β_1 or β_2 receptors or rat D_3 receptors (all baculovirus expression); HeLa cells expressing human recombinant 5-HT_6 receptors; CHO cells expressing human recombinant neurokinin (NK) NK_1, NK_2 or NK_3 receptors; CHO cells expressing human adenosine (A) A_1 receptors. Other membrane preparations purchased from Perkin Elmer NEN Life Science Products were: Madin–Darby canine kidney (MDCK) cells expressing human recombinant norepinephrine transporter; HEK293 cells expressing human adenosine A_{2A} or A_3 receptors; CHO cells expressing human M_1 and M_2 receptors; HEK293 cells expressing human cannabinoid$_1$ receptors; CHO-K1 cells expressing human recombinant $D_{4.4}$ receptors or dopamine transporter; CHO-K1 cells expressing human recombinant opiate δ, μ and κ receptors.

2.3. Membrane preparation

Total rat brain (minus cerebellum) membranes were purchased from ABS, Delaware, USA and prepared according to the following protocol. Male Wistar albino rats (250–300 g) were decapitated, the brains removed, the cerebellar cortices dissected out and the rest of the brains homogenized in 10 vol of ice-cold 50 mM Tris–HCl buffer, pH 7.7, for 30 s. The homogenate was centrifuged at $1000 \times g$ for 10 min, the supernatant collected and centrifuged at $35{,}000 \times g$ for 10 min. The pellet was resuspended in buffer and washed by four further 10-min centrifugations at $35{,}000 \times g$. The final

Table 1
Radioligand binding assays

Receptor, species	Radioligand	Cell/tissue
A_1, human	^{3}H DPCPX	CHO
A_{2A}, human	^{3}H CGS21680	HEK293
A_3, human	^{125}I AB-MECA	HEK293
α_1 Adrenoceptor, rat[a]	^{125}I BE2254	Cortex
α_{2A} Adrenoceptor, human[a]	^{3}H RX821002	CHO
α_{2B} Adrenoceptor, human[a]	^{3}H RX821002	CHO
α_{2C} Adrenoceptor, human[a]	^{3}H RX821002	CHO
AMPA glutamate, rat	^{3}H AMPA	Brain
β_1 Adrenoceptor, human	^{3}H CGP12177	Sf9
β_2 Adrenoceptor, human	^{3}H CGP12177	Sf9
Benzodiazepine, rat	^{3}H Flunitrazepam	Brain
Calcium channel (L) dihydropyridine, rat	^{3}H PN-200-110	Cortex
Calcium channel (N) dihydropyridine, rat	^{125}I Tyr^{22}-ω-conotoxin GVIA	Brain
Cannabinoid$_1$, human	^{3}H CP55940	HEK293
CCK_A, human	^{3}H L-354,718	NIH3T3
CCK_B, human	^{3}H L-365,260	NIH3T3
$CRF_{2\alpha}$, human	^{125}I Sauvagine	CHO
D_1, human[a]	^{3}H SCH-23390	Sf9
D_{2A}, human[a]	^{3}H Spiperone	HEK293
D_3, rat recombinant[a]	^{3}H Spiperone	Sf9
$D_{4.4}$, human[a]	^{3}H Spiperone	CHO-K1
Dopamine transporter, human	^{3}H WIN35428	CHO-K1
5-HT_{1A}, human[a]	^{3}H 8-OH DPAT	CHO-K1
5-HT_{1B}, human[a]	^{3}H 5-HT	CHO
5-HT_{2A} human[a]	^{3}H Ketanserin	CHO
5-HT_{2C}, human[a]	^{3}H Mesulergine	CHO-K1
5-HT_6, human[a]	^{3}H LSD	HeLa
$GABA_A$, rat	^{3}H Muscimol	Cortex
H_1, rat	^{3}H Pyrilamine	Brain
Kainate glutamate, rat	^{3}H Kainic acid	Brain
M_1, human	^{3}H *N*-methyl-scopolamine	CHO
M_2, human	^{3}H *N*-methyl-scopolamine	CHO
NMDA glycine, rat	^{3}H MDL 105,519	Cortex
NMDA channel, rat	^{3}H MK801	Cortex
NMDA glutamate, rat	^{3}H CGP 39653	Cortex
Noradrenaline transporter, human	^{3}H Nisoxetine	MDCK
NK_1, human	^{3}H Sar^{9}SP	CHO
NK_2, human	^{3}H SR48,968	CHO
NK_3, human	^{125}I NKB	CHO
Opiate δ, human	^{3}H DPDPE	CHO
Opiate κ, human	^{3}H Naloxone	CHO
Opiate μ, human	^{3}H Naloxone	CHO-K1

[a] Denotes receptors to which iloperidone and its metabolites actively bind.

pellet was resuspended in buffer (2 ml/brain) and aliquots (2 ml) were frozen and stored at −80 °C. Prior to use, the membrane suspension was thawed quickly at 37 °C, centrifuged at 35,000 × *g* for 10 min, washed once by suspension in the assay buffer and recentrifuged. The final pellet was resuspended and homogenized in the assay buffer to give the desired membrane concentration.

Rat brain cortex membranes were also purchased from ABS and prepared according to the following protocol. The animals were decapitated, the brains removed, the cerebral cortices dissected and homogenized in 10 vol of ice-cold 0.32 M sucrose, containing $MgCl_2$ (1 mM) and K_2HPO_4 (1 mM). The homogenate was centrifuged at 1000 × *g*, the pellet discarded and the centrifugation repeated. The supernatants were pooled and centrifuged at 18,000 × *g* for 15 min. The pellet was osmotically shocked in 10 vol of H_2O and kept on ice for 30 min. The suspension was centrifuged at 39,000 × *g*, the pellet resuspended in Krebs–Henseleit buffer, pH 7.4, containing 20 mM Tris and stored for 2 days at −20 °C. The membranes were thawed at 20–23 °C, washed three times with Krebs–Henseleit buffer by centrifugation at 18,000 × *g* for 15 min, incubated at 4 °C overnight and washed again three times. The final pellet was resuspended in 20 ml of the same buffer. Aliquots were frozen and stored in liquid nitrogen. Prior to use, the membrane suspension was thawed quickly at 37 °C and washed three times by centrifugation at 18,000 × *g* for 15 min.

Cell membranes from CHO cell lines, expressing human recombinant α_{2A}, α_{2B}, α_{2C} and corticotropin-releasing fac-

Table 2
Methodological details for receptor assays

Receptor	Incubation buffer	Time/temperature	Radioligand	Radioligand concentration/K_d	Nonspecific binding[a]	$pK_i \pm$ S.E. for reference compound
α_1 Adrenoceptor, rat	50 mM Tris, 154 mM NaCl, pH 7.5	60 min at 37°	^{125}I BE2254	40 nM/0.17 nM	100 μM phentolamine	Prazosin, 8.92 ± 0.06
α_{2A} Adrenoceptor, human	50 mM Tris, 1 mM EDTA, 140 mM NaCl, pH 7.5	30 min at 22 °C	^{3}H RX821002	2 nM/0.5 nM	10 μM RX821002	RX821002, 9.23 ± 0.03
α_{2B} Adrenoceptor, human	50 mM Tris, 1 mM EDTA, 140 mM NaCl, pH 7.5	30 min at 22 °C	^{3}H RX821002	2 nM/1.55 nM	10 μM RX821002	RX821002, 8.45 ± 0.09
α_{2C} Adrenoceptor, human	50 mM Tris, 1 mM EDTA, 140 mM NaCl, pH 7.5	30 min at 22 °C	^{3}H RX821002	2 nM/0.4 nM	10 μM RX821002	RX821002, 9.23 ± 0.04
Dopamine D_1, human	50 mM Tris, 5 mM EDTA, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 5 mM KCl, pH 7.4	90 min at 27 °C	^{3}H SCH23390	1.6 nM/2.75 M	1 μM (+)butaclamol	(+)Butaclamol, 8.39 ± 0.05
Dopamine D_{2A}, human	50 mM Tris, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4	60 min at 22 °C	^{3}H Spiperone	2 nM/0.14 nM	10 μM (+)butaclamol	(+)Butaclamol, 8.46 ± 0.03
Dopamine D_3, rat recombinant	50 mM Tris, 5 mM EDTA, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 120 mM NaCl, 5 mM KCl, pH 7.4	60 min at 27 °C	^{3}H Spiperone	0.4 nM/0.75 nM	10 μM haloperidol	(+)Butaclamol, 8.1 ± 0.05
Dopamine $D_{4.4}$, human	50 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, pH 7.4	60 min at 25 °C	^{3}H Spiperone	0.7 nM/0.16 nM	2.5 μM haloperidol	(+)Butaclamol, 6.89 ± 0.01
Histamine H_1, rat	50 mM Tris, pH 7.4	30 min at 22 °C	^{3}H Pyrilamine	5 nM/4.52 nM	50 μM pyrilamine	Pyrilamine, 8.68 ± 0.03
5-HT_{1A}, human	50 mM Tris, 2.5 mM $MgCl_2$, 2.5 mM EDTA, pH 7.4	30 min at 22 °C	^{3}H 8-OH-DPAT	1 nM/0.17 nM	10 μM 8-OH-DPAT	8-OH-DPAT, 9.58 ± 0.07
5-HT_{1B}, human	50 mM Tris, 12.5 mM $MgCl_2$, 1 mM EDTA, 0.1% ascorbic acid, pH 7.4	60 min at 22 °C	^{3}H 5-HT	1 nM/1.52 nM	5 μM 5-HT	5-HT, 8.72 ± 0.03
5-$HT_{2A,}$ human	50 mM Tris, pH 7.7	15 min at 22 °C	^{3}H Ketanserin	0.5 nM/0.76 nM	10 μM ketanserin	Ketanserin, 8.59 ± 0.14
5-HT_{2C}, human	50 mM Tris, 0.1% ascorbic acid, 10 μM Pargylline, pH 7.7	60 min at 22 °C	^{3}H Mesulergine	1 nM/3.14 nM	1 μM mesulergine	Mesulergine, 8.83 ± 0.05
5-HT_6, human	50 mM Tris, 0.5 mM EDTA, 10 mM $MgSO_4$, pH 7.4	60 min at 27 °C	^{3}H LSD	3 nM/6.25 nM	100 μM 5-HT	Methiothepin, 10.13 ± 0.14

[a] Nonspecific binding was between 5% and 12%.

tor 2α ($CRF_{2\alpha}$), were generated in the research laboratories of Novartis Pharma and were thawed and homogenized just before the assay.

2.4. Radioligand binding assays

2.4.1. 96-well microtiter plate filtration assay

In general, the membrane preparations were homogenized after thawing and pretreated as required.

Individual radioligand binding assays for different receptors were performed as generally outlined by Herz et al. (1997) with minor modifications as required. The binding studies were performed in 96-well plates (Dynatech, Billinghurst, UK) in a total volume of 250 μl, consisting of the radioligand, drug (iloperidone or metabolite) and membrane preparation (cells or rat brain membranes) diluted in appropriate buffer. Nonspecific binding was determined in the presence of an appropriate drug specific for the receptor under study. The plates were incubated at equilibrium for a specified time, as determined by kinetic experiments, for each receptor assay. Reactions were terminated by flash filtration and inverse transfer to 96-well filter plates [Perkin Elmer/Packard Bioscience, Meriden, CT, USA 96-well cell harvester, filter plates GFC, coated with plasminogen activator inhibitor (PAI) as necessary]. The plates were dried for 30 min at 56 °C and sealed at the bottom with an adhesive sheet (Topseal; Perkin Elmer/Packard Bioscience). Subsequently, 50 μl of scintillation fluid (Microscint-20; Perkin Elmer/Packard Bioscience) was added to each well, the plates sealed on top and the radioactivity counted in a 96-well plate counter (Topcount; Perkin Elmer/Packard Bioscience). The experiments were performed in triplicate with duplicate measurements.

2.5. Data analysis

A standard data reduction algorithm was used to calculate percent specific binding in the presence of the test compound as follows:

$$\frac{[B - \mathrm{NSP}]}{[T - \mathrm{NSP}]} \times 100$$

where: B = binding in the presence of test compound, NSP = nonspecific binding in the presence of excess inhibitor and T = total binding.

IC_{50} values were derived (where feasible) from a four-parameter logistic fit and were converted to pK_i values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

High affinity was arbitrarily defined as $pK_i > 7.7$, moderate affinity as pK_i 6.5–7.7 and low affinity as $pK_i < 6.5$. Affinities of $pK_i \leq 5.0$ were considered negligible (weak).

3. Results

The metabolite P88-8991 primarily bound to the serotonin 5-HT_{2A} receptor (pK_i 9.56), the adrenergic α_1 and α_{2C} receptors (8.08 and 7.79, respectively) and dopamine D_2 receptors (7.80). Furthermore, it had intermediate affinity for other monamine receptors, such as the adrenergic α_{2B}, dopamine D_1, D_3 and $D_{4.4}$, histamine H_1, 5-HT_{1B} and 5-HT_{2C} receptors. Affinities below 6.5 were noted for the α_{2A}, 5-HT_{1A} and 5-HT_6 receptors. The affinity for all other receptors (e.g. adenosine, etc.) was below 10 μM ($pK_i \leq 5$).

With the exception of the 5-HT_{2A} receptors ($pK_i = 8.15$), P95-12113 did not have a high affinity for any of the receptors tested. It had intermediate affinity for the adre-

Table 3
Receptor binding profile for P88-8991, P95-12113 and iloperidone (pK_i)[a]

	P88-8991		P95-12113		Iloperidone	
Receptor, species	Mean	S.E.	Mean	S.E.	Mean	S.E.[a]
α_1 Adrenoceptor, rat	8.08[b]	0.11	7.67[c]	0.02	8.36[b]	0.11
α_{2A} Adrenoceptor, human	6.44[d]	0.02	6.42[d]	0.02	6.79[c]	0.01
α_{2B} Adrenoceptor, human	7.22[c]	0.02	7.08[c]	0.05	6.79[c]	0.11
α_{2C} Adrenoceptor, human	7.79[b]	0.10	7.32[c]	0.09	7.79[b]	0.04
Dopamine D_1, human	7.02[c]	0.01	5.94[d]	0.01	7.37[c]	0.01
Dopamine D_{2A}, human	7.80[b]	0.02	~5		7.67[c]	0.06
Dopamine D_3, rat recombinant	7.17[c]	0.04	6.11[d]	0.05	7.14[c]	0.03
Dopamine $D_{4.4}$, human	7.45[c]	0.05	<5		7.67[c]	0.03
Histamine H_1, rat	7.58[c]	0.20	~5		6.36[d]	0.27
5-HT_{1A}, human	6.37[d]	0.01	~5		6.93[c]	0.07
5-HT_{1B}, human	7.29[c]	0.03	<5		7.05[c]	0.05
5-HT_{2A} human	9.56[b]	0.21	8.15[b]	0.03	9.95[b]	0.27
5-HT_{2C}, human	7.16[c]	0.05	~5		6.60[c]	0.22
5-HT_6, human	6.11[d]	0.03	5.59[d]	0.18	7.20[c]	0.07

[a] Hill coefficients were between 0.78 and 1.00.
[b] High affinity: $pK_i > 7.7$.
[c] Moderate affinity: pK_i 6.5–7.7.
[d] Low affinity: $pK_i < 6.5$.

nergic α_1, α_{2B} α_{2C} receptors, and low affinity ($pK_i < 6.5$) for adrenergic α_{2A}, dopamine D_1 and D_3 and 5-HT_6 receptors. Affinities for all the other receptors tested were weak ($pK_i \leq 5$).

The parent compound, iloperidone, was tested in parallel with its metabolites (Table 3). The affinity data for some of the receptors detailed differed from previous reported results (Kalkman et al., 2001; Kongsamut et al., 1996), as these were measured at different times and in different laboratories. However, in agreement with previous reported data (Kongsamut et al., 1996), iloperidone bound with high affinity to serotonin 5-HT_{2A} and adrenergic α_1 and α_{2C} receptors.

In general, P95-12113 had a much weaker affinity for the receptors tested compared to that of iloperidone (Table 3). However, P88-8991 displayed a more similar binding profile to the parent compound, with the exception of the 5-HT_6 and H_1 receptors, where the metabolite had a lower affinity for the 5-HT_6 and a higher affinity for the H_1 receptors.

4. Discussion

Classical antipsychotic drugs, like the phenothiazines (e.g., chlorpromazine and fluphenazine) or butyrophenones (e.g., haloperidol and pimozide), are all potent dopamine D_2 receptor antagonists (Meltzer et al., 1989). Second-generation antipsychotic compounds are generally characterized by a lower affinity to D_2 receptors and thereby display reduced propensity to induce EPS and hyperprolactinemia (Kapur and Seeman, 2001).

D_2/D_3 receptor antagonists are known to improve positive symptoms of schizophrenia, presumably by preventing overactivation of D_2 and D_3 receptors in the nucleus accumbens (Davis et al., 1991; Willner, 1997). Unfortunately, these compounds do not (or, do not sufficiently) correct the dopaminergic hypoactivity in the frontal cortex (Davis et al., 1991). Clozapine, which is a weak D_2 receptor antagonist (Schotte et al., 1996), effectively increases prefrontal dopamine and noradrenaline levels (Moghaddam and Bunney, 1990; Yamamoto et al., 1994; Westerink et al., 2001). Interestingly, clozapine is often effective in patients with schizophrenia who are treatment-refractory to other D_2/D_3 antagonists (Kane et al., 1988; Conley et al., 1999; Taylor and Duncan-McConnell, 2000). Therefore, it is evident that clozapine must possess one or more additional mechanisms of action. In this respect, blockade of the α_2 adrenoceptor is attracting increasing attention (Lindström, 2000). Idazoxan, a α_2 adrenoceptor antagonist, induced a therapeutic effect in patients with schizophrenia who were treatment-resistant to the D_2 antagonist, fluphenazine (Litman et al., 1996). Idazoxan also potentiated the effect of raclopride in a conditioned avoidance paradigm in rats (Hertel et al., 1999). Moreover, idazoxan induced a robust increase in prefrontal extracellular dopamine levels (Hertel et al., 1999). On the basis of these data, one would predict that blockade of α_2 receptors will ameliorate the cognitive deficits arising from dopaminergic hypofrontality. The α_{2C} subtype of α_2 receptors is also attracting particular attention. Experiments with genetically altered mice have shown that overexpression of α_{2C} adrenoceptors is associated with behaviors that are believed to be representative of anxiety, depression and cognitive impairment in animal models (Björklund et al., 1998; Sallinen et al., 1999). Additionally, a wide range of other monoamine receptors, particularly the α_1 adrenoceptors and 5-HT_{2A} receptors, have been implicated in the pharmacological profile of many of the new atypical antipsychotics (Kongsamut et al., 1996; Schotte et al., 1996; Busatto and Kerwin, 1997).

4.1. Receptor affinity of iloperidone

Iloperidone has affinity for dopamine D_2 and D_3 receptors similar to that seen with other second-generation compounds such as olanzapine and ziprasidone. In addition, iloperidone also binds to 5-HT_{2A}, α_1- and α_{2C} adrenoceptors (Kalkman et al., 2001). Theoretically, this receptor profile could lead to an enhanced therapeutic effect in schizophrenia and other psychotic disorders involving depression, such as schizoaffective disorder and bipolar disorder.

4.2. Metabolism of iloperidone

The metabolic fate of iloperidone has been studied in both animals and humans (Chesson et al., 1991; Mutlib and Klein, 1998; Mutlib et al., 1995). Three metabolic pathways participate in the biological degradation of iloperidone. In a (reversible) reductive step, the ketone group in iloperidone is reduced to form the hydroxy-metabolite, P88-8991.

Secondly, cleavage of the aromatic methoxy-ether results in the phenol metabolite, P89-9124, which is a major metabolite in rodents but only a minor one in humans. The third pathway involves oxidation of the ethanone part of the molecule, initially resulting in the α-hydroxy ketone, P94-11840 (Chesson et al., 1991). In healthy human volunteers, an additional metabolite, P95-12113, has been found in plasma and urine that results from further oxidation and decarboxylation of the α-hydroxy keto metabolite (Novartis Pharma, data on file). Although this metabolite has not been observed in previous animal studies, it lies on a metabolic pathway common to all species studied, and may have been overlooked by the analytical methodology employed in earlier preclinical studies. In humans, P88-8991 and P95-12113 are the major circulating metabolites in the plasma following ingestion of iloperidone. Studies have shown that at a dose of 16 mg/day, steady state C_{max} values for iloperidone, P88-8991 and P95-12113 are 20, 25 and 40 ng/ml, respectively (Novartis Pharma, data on file). Depending on the inherited pattern of chromosome *P*450 isoforms, P88-8991 and/or P95-12113 may reach higher plasma levels than the parent compound (Novartis Pharma, data on file).

4.3. Receptor affinity of P88-8991 and its contribution to the clinical profile of iloperidone

P88-8991 shows high affinity for serotonin 5-HT_{2A}, adrenergic α_1, α_{2C} and dopamine D_2 receptors similar to that of iloperidone, with equal affinity for the α_{2C} and D_2 receptors. The results show that P88-8991 has an affinity for the 5-HT_{2A} receptors ($pK_i = 9.56$) that is only 2.5 times less than that of iloperidone ($pK_i = 9.95$); and its affinity for D_2 receptors ($pK_i = 7.80$) is comparable to that of the parent compound ($pK_i = 7.67$). These data also show that the affinity of P88-8991 for 5-HT_{2A} receptors is higher than that for D_2 receptors. Antipsychotics with a higher affinity for 5-HT_{2A} receptors than D_2 receptors have been suggested to have a reduced propensity to induce EPS and may have efficacy against the negative and cognitive symptoms of schizophrenia (Corbett et al., 1997). The high affinity of P88-8991 for the α_{2C} receptor ($pK_i = 7.79$) suggests that it may well contribute to the antipsychotic efficacy of iloperidone, especially with regard to cognitive dysfunction and depressive symptoms. Similar to iloperidone, P88-8991 displays a low affinity for the α_{2A} receptors, which would infer that the metabolite is also unlikely to be involved in the induction of convulsions, the occurrence of which has been linked to a blockade of α_{2A} receptors (Janumpalli et al., 1998).

The extent to which P88-8891 will contribute to the efficacy and tolerability profiles of iloperidone will depend largely on its pharmacokinetic profile. Clinical Phase I pharmacology studies have shown that the half-life ($t_{1/2}$) for iloperidone is approximately 17 h. Despite this long $t_{1/2}$, there were no signs of accumulation of iloperidone as evidenced by the predictability of steady state area under the plasma concentration-versus-time curve (AUC) from the single-dose $AUC_{0-\infty}$. P88-8891 also demonstrates a kinetic profile very similar to iloperidone in patients (Novartis Pharma, data on file).

Preclinical experiments, such as the mouse apomorphine-climbing test, the rat self-stimulation and the pole climb avoidance task, indicate antipsychotic activity of P88-8991 and confirm that this metabolite crosses the blood–brain barrier (Novartis Pharma, data on file). Thus, considering the pharmacodynamic and pharmacokinetic profile, it is expected that P88-8991 will contribute to, but not alter, the therapeutic profile of iloperidone.

4.4. Receptor affinity of P95-12113 and its contribution to the clinical profile of iloperidone

P95-12113 shows high affinity only for 5-HT_{2A} receptors. It has moderate affinity for adrenergic α_1, α_{2B} and α_{2C} receptors and low affinity for adrenergic α_{2A}, D_1, D_3 and 5-HT_6 receptors. P95-12113 shows negligible affinity for all the other receptors tested. As such, it is the metabolite that exhibits the lowest of receptor affinities, and furthermore, these affinities are considerably weaker than those of the parent compound.

In preclinical pharmacokinetic studies, the metabolite P95-12113 is not detectable in brain tissue and therefore will only contribute to peripheral side effects. As P95-12113 binds to the same set of receptors as iloperidone but with a significantly lower affinity, we expect no additional side effects to arise from this peripheral metabolite.

5. Conclusion

In combination with results from animal studies, the present radioligand binding studies indicate that the peripheral metabolite P95-12113 is unlikely to add to the therapeutic profile of iloperidone. However, the receptor binding profile of P88-8991 suggests that it should contribute to an enhanced clinical profile and tolerability of iloperidone. These data support iloperidone, a novel psychotropic agent, as a compound with therapeutic efficacy and low propensity for side effects.

References

Björklund, M., Sirviö, J., Puoliväli, J., Sallinen, J., Jäkälä, P., Scheinin, M., Kobilka, B.K., Riekkinen Jr., P., 1998. α_{2C}-Adrenoceptor-overexpressing mice are impaired in executing non-spatial and spatial escape strategies. Mol. Pharmacol. 5, 569–576.

Borison, R.L., Huff, F.J., Griffiths, L., Ramaswamy, R., Shipley, J.E., 1996. Efficacy of 4 mg/day and 8 mg/day iloperidone (HP873) administered to schizophrenic patients for 42 days. Psychopharmacol. Bull. 32, 416.

Busatto, G.F., Kerwin, R.W., 1997. Perspectives on the role of serotonergic mechanisms in the pharmacology of schizophrenia. J. Psychopharmacol. 11 (1), 3–12.

Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K_1) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Chesson, S.M., Hsu, R.S., Dileo, E.M., Strupczewski, J., 1991. Pharmacokinetics of HP 873, a potential atypical antipsychotic, in rats and dogs. ASPET Conference, San Diego, CA.

Conley, R.R., Tamminga, C.A., Kelly, D.L., Richardson, C.M., 1999. Treatment-resistant schizophrenic patients respond to clozapine after olanzapine non-response. Biol. Psychiatry 46, 73–77.

Corbett, R., Griffiths, L., Shipley, J.E., Shukla, U., Strupczewski, J.T., Szczepanik, A.M., 1997. Iloperidone: preclinical profile and early clinical evaluation. CNS Drug Rev. 3, 120–147.

Davidson, M., Brecher, M., Kahn, R., Stern, R., Macaluso, J., 1994. Iloperidone (HP 873): a study of safety and tolerance in schizophrenic inpatients. Psychopharmacol. Bull. 30, 664.

Davis, K.L., Kahn, R.S., Ko, G., Davidson, M., 1991. Dopamine in schizophrenia: a review and reconceptualization. Am. J. Psychiatry 148, 1474–1486.

Deutch, A.Y., Moghaddam, B., Innis, R.B., Krystal, J.H., Aghajanian, G.K., Bunney, B.S., Charney, D.S., 1991. Mechanisms of action of atypical antipsychotic drugs. Implications for novel therapeutic strategies for schizophrenia. Schizophr. Res. 4, 121–156.

Hertel, P., Fagerquist, M.V., Svensson, T., 1999. Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by α_2 adrenoceptor blockade. Science 286, 105–107.

Herz, J.M., Thomsen, W.J., Yarbrough, G.G., 1997. Molecular approaches to receptor targets for drug discovery. J. Rec. Signal Transduction Res. 17, 671–776.

Janumpalli, S., Butler, L.S., MacMillan, L.B., Limbird, L.E., McNamara, J.O., 1998. A point mutation (D79N) of the alpha$_{2A}$ adrenergic receptor abolishes the antiepileptogenic action of endogenous norepinephrine. J. Neurosci. 15 (18), 2004–2008.

Kalkman, H.O., Subramanian, N., Hoyer, D., 2001. Extended radioligand binding profile of iloperidone: a broad spectrum dopamine/serotonin/norepinephrine antagonist for the management of psychotic disorders. Neuropsychopharmacology 25, 904–914.

Kane, J.M., Honigfeld, G., Singer, J., Meltzer, H., 1988. The Clozaril collaborative study group. Clozapine for the treatment-resistant schizophrenic. Arch. Gen. Psychiatry 45, 789–796.

Kapur, S., Seeman, P., 2001. Does fast dissociation from the dopamine D_2 receptor explain the action of atypical antipsychotics?: a new hypothesis. Am. J. Psychiatry 158, 360–369.

Kongsamut, S., Roehr, J.E., Cai, J., Hartman, H.B., Weissensee, P., Kerman L.L., Tang, L., Sandrasagra, A., 1996. Iloperidone binding to human and rat dopamine and 5-HT receptors. Eur. J. Pharmacol. 317, 417–423.

Lindström, L.H., 2000. Schizophrenia, the dopamine hypothesis and α_2-adrenoceptor antagonists. Trends Pharmacol. Sci. 21, 198–199.

Litman, R.E., Su, T.P., Potter, W.Z., Hong, W.W., Pickar, D., 1996. Idazoxan and response to typical neuroleptics in treatment-resistant schizophrenia. Comparison with the atypical neuroleptic, clozapine. Br. J. Psychiatry 168, 571–579.

Meltzer, H.Y., Matsubara, S., Lee, J.C., 1989. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pK_i values. J. Pharmacol. Exp. Ther. 251, 238–246.

Moghaddam, B., Bunney, B.S., 1990. Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study. J. Neurochem. 54, 1755–1760.

Mutlib, A.E., Klein, J.T., 1998. Application of liquid chromatography/mass spectrometry in accelerating the identification of human liver cytochrome *p*450 isoforms involved in the metabolism of iloperidone. J. Pharmacol. Exp. Ther. 286, 1285–1293.

Mutlib, A.E., Strupczewski, J.T., Chesson, S.M., 1995. Application of hyphenated LC/NMR and LC/MS techniques in rapid identification of in vitro and in vivo metabolites of iloperidone. Drug Metab. Dispos. 23 (9), 951–964.

Sallinen, J., Haapalinna, A., MacDonald, E., Viitamaa, T., Lähdesmäki, J., Rybnikova, E., Pelto-Huikko, M., Kobilka, B.K., Scheinin, M., 1999. Genetic alteration of the α_2-adrenoceptor subtype C in mice affects the development of behavioral despair and stress-induced increase in plasma corticosterone levels. Mol. Psychiatry 4 (5), 443–452.

Schotte, A., Janssen, P.F.M., Gommeren, W., Luyten, W.H.M.L., van Gompel, P., Lesage, A.S., De Loore, K., Leysen, J.E., 1996. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology 124 (1–2), 57–73.

Svensson, T.H., Mathé, J.M., Andersson, J.L., Nomikos, G.G., Hildebrandt B.E., Marcus, M., 1995. Mode of action of atypical neuroleptics in relation to the phencyclidine model of schizophrenia: role of 5-HT$_2$ receptor and α_1-adrenoceptor antagonism. J. Clin. Psychopharmacol. 15 (Suppl. 1), 11S–18S.

Taylor, D.M., Duncan-McConnell, D., 2000. Refractory schizophrenia and atypical antipsychotics. J. Psychopharmacol. 14 (4), 409–418.

Westerink, B.H.C., Kawahara, Y., De Boer, P., Geels, C., De Vries, J.B., Wikstrom, H.V., Van Kalkeren, A., Van Vliet, B., Kruse, C.G., Long, S.K., 2001. Antipsychotic drugs classified by their effects on the release of dopamine and noradrenaline in the prefrontal cortex and striatum. Eur. J. Pharmacol. 412 (2), 127–138.

Willner, P., 1997. The dopamine hypothesis of schizophrenia: current status, future prospects. Int. Clin. Psychopharmacol. 12 (6), 297–308.

Yamamoto, B.K., Pehek, E.A., Meltzer, H.Y., 1994. Brain region effects of clozapine on amino acid and monoamine transmission. J. Clin. Psychiatry 55 (Suppl. B), 8–14.