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ORIGINAL INVESTIGATION

The antipsychotic potential of l-stepholidine—a naturally occurring dopamine receptor D_1 agonist and D_2 antagonist

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Abstract
Rationale l-Stepholidine, a dopamine D_2 antagonist with D_1 agonist activity, should in theory control psychosis and treat cognitive symptoms by enhancing cortical dopamine transmission. Though several articles describe its impact on the dopamine system, it has not been systematically evaluated and compared to available antipsychotics.
Materials and methods We examined its in vitro interaction with dopamine D_2 and D_1 receptors and compared its in vivo pharmacokinetic profile to haloperidol (typical) and clozapine (atypical) in animal models predictive of antipsychotic activity.
Results In vitro, l-stepholidine showed significant activity on dopamine receptors, and in vivo, l-stepholidine demonstrated a dose-dependent striatal receptor occupancy (RO) at D_1 and D_2 receptors (D_1 9–77%, 0.3–30 mg/kg; D_2 44–94%, 1–30 mg/kg), though it showed a rather rapid decline of D_2 occupancy related to its quick elimination. In tests of antipsychotic efficacy, it was effective in reducing amphetamine- and phencyclidine-induced locomotion as well as conditioned avoidance response, whereas catalepsy and prolactin elevation, the main side effects, appeared only at high D_2RO (>80%). This preferential therapeutic profile was supported by a preferential immediate early gene (Fos) induction in the nucleus accumbens over dorsolateral striatum. We confirmed its D_1 agonism in vitro, and then using D_2 receptor, knockout mice showed that l-stepholidine shows D_1 agonism in the therapeutic dose range.

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Conclusions Thus, l-stepholidine shows efficacy like an "atypical" antipsychotic in traditional animal models predictive of antipsychotic activity and shows in vitro and in vivo D_1 agonism, and, if its rapid elimination does not limit its actions, it could provide a unique therapeutic approach to schizophrenia.

Keywords l-Stepholidine · Antipsychotic · D_1 and D_2 receptor occupancy · Schizophrenia · Animal models

Introduction

Schizophrenia is a devastating, lifelong condition characterized by disordered thinking, perceptual abnormalities, and a number of cognitive difficulties including apathy and disturbance of executive function. While first and second generation antipsychotics targeting the D_2 receptors have had a major impact on psychosis, they have had minimum impact on the negative, cognitive, and social consequences of these disorders (Kane and Malhotra 2003). There is also evidence for a decreased level of D_1 receptor-like binding in the prefrontal cortex of drug-naive patients with schizophrenia, and this has been correlated with the severity of negative symptoms and cognitive dysfunction in these patients (Okubo et al. 1997). It has also been shown that chronic D_2 receptor blockade in non-human primates results in downregulation of D_1 receptors in the prefrontal cortex and consequently produces severe impairment in working memory (Castner et al. 2000). Hence, modulation of D_1 receptors in the prefrontal cortex has been postulated to play an important role in working memory and thus is an important target in treating cognitive dysfunction in schizophrenia (Goldman-Rakic et al. 2000; Abi-Dargham et al. 2002).

In this light, l-stepholidine, a tetrahydroberberine alkaloid isolated from the Chinese herb *Stephania intermedia*, is particularly interesting (Jin et al. 2002). l-Stepholidine has been shown to have high affinity for dopamine D_1 and modest affinity for D_2 receptors (K_i of 13 and 85 nM using [^{3}H]SCH23390 and [^{3}H]spiperone, respectively, in calf striatal tissue; Jin 1987; Jin et al. 2002). In functional assays, it has been characterized as an *agonist* at the dopamine D_1 receptor, as an *antagonist* at the dopamine D_2 receptor (Dong et al. 1997a; Dong et al. 1997b), and is fairly selective to the dopaminergic system (Jin and Sun 1995). Also, a search of the online database of the National Institute of Mental Health (NIMH), USA's Psychoactive Drug Screening Program (PDSP) revealed l-stepholidine to have high affinity mostly for dopaminergic receptors (D_1 5.9; D_2 974.3; D_3 30.1; D_4 3748; D_5 4.4 K_i in nanomolar), and the other receptors for which appreciable affinity is exhibited are serotonergic 5-HT_{1A} (K_i 143.4 nM) and Sigma 2 (K_i 53.4 nM) receptors. Hence, l-stepholidine appears to be a potentially attractive therapeutic option for schizophrenia, as its D_2 receptor antagonism could bestow standard antipsychotic activity, while its D_1 agonism, by enhancing cortical dopaminergic transmission, could lead to superior efficacy against cognitive and negative symptoms (Lidow et al. 1991; Hall et al. 1994; Abi-Dargham 2004).

Jin and colleagues have studied l-stepholidine's pharmacology in animal models and have shown that l-stepholidine acts as a D_2 receptor antagonist in in vivo animal models (Jin et al. 1992; Zou et al. 1996; Jin et al. 2002; Ellenbroek et al. 2006), but evidence for l-stepholidine's D_1 actions mainly comes from its in vitro binding to D_1 receptors (Dong et al. 1997a), and behavioral evidence of its D_1 agonism is weak and mainly comes from studies on dopamine receptor supersensitive states (Jin et al. 1992; Liu et al. 1999). There are a number of shortcomings in the available data: first, direct measures of D_1 agonism are lacking since in most animal models the effect of D_1 agonism is confounded by concomitant D_2 antagonism; second, systematic comparison to existing typical and atypical antipsychotics at relevant and comparable doses is not available; third, l-stepholidine's use in previous models has been carried out at different dose ranges (e.g., 1–16 mg/kg for the startle and paw test paradigm; 10–40 mg/kg for Fos expression; 2–40 mg/kg for catalepsy and stereotypy experiments) without anchoring it to a putative antipsychotic dosing range (Zhang et al. 1997; Mo et al. 2005; Ellenbroek et al. 2006); and finally, we observed that l-stepholidine had a particularly fast in vivo elimination time course, leading us to examine its pharmacokinetic and receptor occupancy properties in detail.

To advance our understanding of l-stepholidine, we first evaluated its in vitro interaction with a number of neurotransmitter receptors and transporters, ion channels, brain/gut peptides, and enzymes not evaluated by NIMH's PDSP. Later, we evaluated its in vitro D_1 agonistic and D_2 antagonistic activity. We compared l-stepholidine to two widely used and clinically studied antipsychotics: haloperidol, a conventional typical antipsychotic drug, and clozapine, the classical atypical drug in terms of their striatal dopamine receptor occupancy (Kapur et al. 2003) as well as their antipsychotic and side effect profiles. Guided by the occupancy studies, the following indices were determined: (a) amphetamine- and phencyclidine-induced hyperlocomotion (AIL/PIL), (b) conditioned avoidance response (CAR), (c) catalepsy (CAT), (d) Fos expression in the striatum, and (e) plasma prolactin levels, as these are commonly used preclinical tests related to antipsychotic activity and side effects (Robertson et al. 1994; Arnt and Skarsfeldt 1998; Natesan et al. 2006). In an effort to tease out the D_1 agonist effects of this dual action compound, we examined its effects on locomotor function

and brain Fos protein expression using D_2 receptor knockout mice. Lastly, to explain a short acting pharmacodynamic profile exhibited by l-stepholidine, we determined the time course of its striatal D_2RO profile and estimated plasma as well as brain tissue levels of a single dose to understand its pharmacokinetic nature.

Materials and methods

Animals

Adult male Sprague–Dawley rats weighing 250–275 g (Charles River Laboratories, Montreal, Canada), housed in pairs under reversed lighting conditions (lights on: 7 pm, lights off: 7 am) were used for the study. $D_2^{-/-}$ mice were generated by interbreeding heterozygotes for the dopamine D_2 gene originally obtained from Jackson Laboratories (Bar Harbor, ME, USA) and were typed by PCR analysis at weaning. They were housed up to a maximum of five mice per cage in normal lighting conditions (lights on: 7 am, lights off: 7 pm). Only male D_2-null mice or wild-type (17–24 g; 8–10 weeks in age) were used. All animal experiments were approved by the CAMH's animal care committee, and experimenters were blinded as far as possible.

Drugs

l-Stepholidine (Calbiochem, San Diego, CA, USA), was dissolved in 30% dimethylformamide (in acidified saline), while haloperidol (Sabex Inc., Boucherville, QC, Canada) and clozapine (ANAWA Trading SA, Wangen, Zurich, Switzerland) were dissolved in 1% glacial acetic acid in saline. SKF81297 and SKF83566 were obtained from Sigma Aldrich®, Canada and dissolved in 30% dimethylformamide (in acidified saline). All drugs were administered subcutaneously (s.c.) in a volume of 1 ml/kg of body weight in rats and in a volume of 10 ml/kg in mice unless specifically mentioned. [^{3}H]SCH23390 and [^{3}H]raclopride (Perkin Elmer Life Sciences, Boston, MA, USA), used as radiotracers in the occupancy studies, were administered intravenously and all reagents used were of analytical grade.

l-Stepholidine's in vitro activity in a broad screen and at dopamine receptors

l-Stepholidine was evaluated in vitro in customized screening assays (Novascreen® Biosciences Corporation, USA), at a single concentration (10^{-7} M), in duplicates, for interaction with a number of neurotransmitter receptors (including cholinergic, GABA, glutamate), transporters (dopamine, norepinephrine, and serotonin), ion channels (including calcium, potassium, and sodium), brain/gut peptides (including angiotensin, neurokinin, and vasopressin), and enzymes (including choline esterase, glutamic acid decarboxylase, and monoamine oxidase), excluding those whose information was available at the PDSP website of NIMH (refer to Electronic Supplementary Information Table 1 for details).

Affinity to D_1 and D_2 receptors was evaluated using HEK-293T cells expressing D_1 or D_2 receptors, generated and maintained in monolayer cultures at 37°C in minimal essential medium supplemented with 10% fetal bovine serum and antibiotics. cDNA encoding for the human D_1 receptor or the long isoform of the human D_2 receptor inserted by PCR into pcDNA3 vector (Invitrogen) was transfected into the cells using Lipofectamine™. The radioligand binding experiments were performed in membranes isolated from cells, 48 h after transfection. While D_1 receptor binding studies were performed with [^{3}H]SCH23390, D_2 receptor binding studies were performed with [^{3}H]raclopride as previously described (So et al. 2005). The competition binding studies were performed in the absence or presence of the non-hydrolyzable GTP analogue, GTPγS, in a concentration of 100 μM. The data were analyzed to fit a one-site or two-site model using least squares regression analysis (Prism, GraphPad). Each experiment was performed in triplicate.

Adenylyl cyclase activity was evaluated by measurement of cyclic AMP accumulation. Cells stably expressing the D_1 receptor or the D_2 receptor at densities of 800–1,000 fmol/mg protein were used. For adenylyl cyclase activation studies, cells expressing the D_1 receptor were treated with varying concentrations of dopamine or l-stepholidine for 20 min, then washed, lysed in 0.1 N HCl, and the supernatant assayed for intracellular cyclic AMP levels using an enzyme-linked immunoassay (Cayman Chemical, Ann Arbor, MI, USA). For the adenylyl cyclase inhibition studies, cells expressing the D_2 receptor were treated with forskolin 1 μM and dopamine 10 μM, together, with increasing concentrations of l-stepholidine for 20 min. Following this, the cells were washed and lysed, and the intracellular cyclic AMP measured as above. Each experiment was performed in triplicate.

Dopamine receptor occupancy experiments

Dose responses for D_1 receptor occupancy (D_1RO) and D_2 receptor occupancy (D_2RO) were determined by administering 7.5 μCi/rat of [^{3}H]SCH23390 or [^{3}H]raclopride diluted in saline in a constant volume of 0.4 ml, intravenously, 30 min before sacrifice. Rats were decapitated, striatal and cerebellar brain tissues were dissected and weighed, and under constant shaking, the tissues were dissolved overnight in a solution of sodium hydroxide Solvable™ (Perkin Elmer, USA, 2 ml per vial). This was

followed by another 24 h of shaking with Aquasure™ scintillation cocktail (Perkin Elmer, USA, 5 ml per vial). Radioactivity (disintegrations per minute, DPM) in the dissolved tissues was measured using fluorescent spectrometry. The binding potential (BP), defined as the ratio of specific (striatal tissue DPM per milligram) to non-specific binding (cerebellar DPM per milligram), was determined. Occupancy induced by the drug was calculated using the formula: % Occupancy = $100 \times (BP_{pooled\ controls} - BP_{drug}/BP_{pooled\ controls})$ (Wadenberg et al. 2000). Five rats were used for each dose level; occupancy curves and the ED_{50} values, if attained, were determined using the non-linear regression equation representing a rectangular hyperbola, using Sigma Plot®.

Amphetamine and phencyclidine-induced locomotion

The effects of l-stepholidine, haloperidol, and clozapine on AIL and PIL were evaluated in locomotor activity boxes (clear Plexiglas—27×48×20 cm, equipped with a row of six photocell beams and a computer to detect photobeam interruptions). Rats were first injected with the drug or vehicle and placed in the locomotor activity boxes for a period of 30 min for habituation. Then, *d*-amphetamine (1.5 mg/kg/s.c.; US Pharmacopoeia, Rockville, MD, USA) or phencyclidine (5 mg/kg/s.c.; Sigma, UK) was administered and locomotor activity was monitored for a period of 60 min. The number of rats at each dose level was five. The ED_{50} value was the dose that was required to inhibit 50% of horizontal locomotor activity counts recorded over a period of 60 min with respect to vehicle and amphetamine/phencyclidine-treated animals and was calculated using linear or non-linear regression, based on the best-fit of the regression curve.

Conditioned avoidance responding

Computer-assisted two-way active avoidance shuttle boxes (Med Associates, VT, USA) were used to train and test the rats. A microswitch system identified the location of the rat in the two-compartment shuttle box. The rats were habituated to the shuttle boxes and were trained for 5 days, and each day consisted of a session of 40 trials. An 80-dB white noise served as a conditioned stimulus, followed 10 s later by a scrambled 0.8-mA shock, serving as the unconditioned stimulus. Rats that moved to the other side of the box within the period of the conditioned stimulus (10 s) were noted as having made an "avoidance" response. Those who escaped the shock in the next 20 s were termed as having "escaped", and those not escaping were termed as "escape failures". Rats achieving greater than 80% avoidance responses were chosen for testing. Animals of each drug group (n=6 for each drug) served as their own controls in a within-subject design, counterbalanced as far as possible for the sequence of drug administration. Animals were tested at 0 (prior to drug administration), 20, 90, 240 min and 24 h after drug administration with an interval of at least 2 days between experiments, and each time point consisted of a 20 trial session. The ED_{50} for CAR, the dose required to produce 50% inhibition of avoidance, was calculated using probit analysis (Finney 1971).

Catalepsy

CAT was evaluated 50 min after drug administration in the same animals used for the D_2 receptor occupancy experiment. Animals were placed on an inclined grid (60°), and the time the animals remained immobile (excluding the first 30 s) was used as an index of CAT (on a scale 0–5 in which time was a square root transformation: 0=0–0.08, 1=0.09–0.35, 2=0.36–0.80, 3=0.81–1.42, 4=1.43–2.24, 5=>2.24 min; Wadenberg et al. 2000). An animal was considered cataleptic with a score greater than or equal to 2, and the ED_{50} values were evaluated using probit analysis (Finney 1971).

Fos immunohistochemistry in rats

Two hours after drug administration, rats were anesthesized using sodium pentobarbital (100 mg/kg i.p.) and their brains removed after transcardial perfusion with saline followed by 4% paraformaldehyde. The brains were post-fixed in 4% paraformaldehyde, transferred to sucrose solutions (10% for 2 h, 20% for 12 h, and 30% for 24 h), and then dried and stored at −80°C until processing. Fos immunoreactive nuclei, labeled with antiserum raised in rabbits against the Fos peptide 4–17 amino acids of human Fos (Oncogene Research Products, Cambridge, MA, USA), were counted within a 400×400-μm grid at a magnification of ×100 in the shell of the nucleus accumbens and dorsolateral striatum (Paxinos and Watson 1986; Robertson et al. 1994). Cell counts were obtained from at least three separate brain sections, for each brain, in at least four subjects per group.

Plasma prolactin measurements

Prolactin levels (nanogram per milliliter) were measured using plasma collected from rats sacrificed for the D_2RO occupancy experiment, 1 h after drug administration, using a rat prolactin enzyme immunoassay kit (ALPCO Diagnostics®, Windham, NH, USA).

D_2 receptor knockout mice—locomotor activity

In order to determine a suitable dose for testing l-stepholidine in D_2 knockout mice, the dose required for catalepsy was determined in the background strain (C57/

BL6) of the D_2 knockout mice. CAT was measured at 30, 60, 90, and 120 min after drug administration (doses of 2.5, 5, and 10 mg/kg and vehicle were tested; n=5 each group) and a dose close to its 1-h ED_{50} was used for further experimentation. Locomotion was measured in locomotor activity boxes similar to the one described earlier, equipped with a row of 11 photocell beams instead of six. Mice (n=7) were allowed to habituate to the locomotor boxes for a period of 30 min and were injected with the vehicle, l-stepholidine, or SKF81297 and assessed for locomotor activity for a period of 120 min. Mice served as their own controls in a within-subject design separated by a 2-day interval between test days. The dose of l-stepholidine, 6 mg/kg, was decided based on the ED_{50} (4.7 mg/kg) for inducing CAT in C57/BL6 mice (the background strain of the D_2 knockout mice).

D_2 receptor knockout mice—Fos immunohistochemistry

Mice in separate groups were administered the vehicle (n=4), l-stepholidine (n=4), SKF81297 (n=4), as well as SKF83566 + l-stepholidine (n=3). SKF83566 was administered 15 min prior to l-stepholidine. Fos immunoreactive nuclei were counted within a 270×270-μm grid at a magnification of ×100 in the medial prefrontal cortex (prelimbic region), shell of the nucleus accumbens, and dorsolateral striatum (Franklin and Paxinos 1997), using the methods similar to the rat experiments described earlier.

Plasma and brain tissue drug levels

A sensitive and reliable assay for the quantification of l-stepholidine (SPD) in rat plasma and brain was used (Odontiadis et al. 2007). Brain regions (prefrontal cortex, striatum, and cerebellum) and plasma from rats treated with l-stepholidine (10 mg/kg s.c.) 20, 40, 60, or 90 min prior to sacrifice were analyzed for drug levels (n=5 rats for each time point). The brain versus plasma drug level was analyzed using non-compartmental methods with statistical moment analysis (Yamaoka et al. 1978). The area under the plasma/tissue concentration–time curve, up to the time of the last quantified concentration ($AUC_{0\text{-tlast}}$), was calculated by the linear trapezoidal method, and due to limited sampling points, especially in the terminal phase, k_e estimated earlier [k_{10}=1.63 h^{-1} for central compartment (plasma); k_{21}=1.78 h^{-1} for peripheral compartment (brain tissue) from a two compartmental model analysis (Zhang et al. 1990)] was used. The value of AUC, extrapolated to infinity ($AUC_{0-\infty}$), was calculated as $AUC_{0-tlast}+C_{last/ke}$, where C_{last} is the last quantified concentration in plasma/tissues. The area under the first moment of the concentration versus time curve, up to the time of the last quantified concentration ($AUMC_{0-tlast}$), was calculated for non-compartmental analysis by the linear trapezoidal method. The value of AUMC, extrapolated to infinity ($AUMC_{0-\infty}$), was calculated as $AUMC_{0-tlast}+t_{last}C_{last/ke}+C_{last}/k_e^2$. The drug distribution index between brain and plasma was determined as the ratio of $(AUC)_{brain}/(AUC)_{plasma}$. The mean transit time (MTT) for plasma as well as brain tissues was calculated as $(AUMC_{0-\infty})/(AUC_{0-\infty})$.

Results

Activity of l-stepholidine in the broad screen and at D_1 and D_2 dopamine receptors

l-Stepholidine, tested at a single concentration (10^{-7} M) in duplicates in the broad screen which included a number of neurotransmitter receptors and transporters, ion channels, brain/gut peptides, and enzymes, showed activity fairly selective to dopaminergic receptors (refer to Electronic Supplementary Information Table 1 for details).

In membranes from cells expressing D_1 receptors, l-stepholidine displaced [^{3}H]SCH23390 binding to reveal two sites with K_i values of 7.6×10^{-9} and 3.0×10^{-7} M, in a proportion of 75:25% (Fig. 1a). Treatment with GTPγS resulted in a marked reduction of the high affinity site revealing a single affinity site with K_i 1.3×10^{-7} M. In membranes from cells expressing D_2 receptors, l-stepholidine displaced [^{3}H]raclopride binding to reveal a single site with K_i 2.0×10^{-9} M (Fig. 1b). Treatment with GTPγS had no effect. These data reveal that the binding of l-stepholidine discriminates between the G protein-linked D_1 receptor with high affinity and the lower affinity site but not with D_2 receptors. The D_1 high affinity site was reduced to a single low affinity site following GTP analogue treatment-induced uncoupling of G protein.

To evaluate the functional response of l-stepholidine at D_1 and D_2 receptors, the effects on adenylyl cyclase activity were assessed. In cells expressing the D_1 receptor, dopamine action resulted in an increase of cyclic AMP accumulation with an EC_{50} of 50±18 nM, as shown by a representative analysis (Fig. 1c). l-Stepholidine also induced a robust increase in cyclic AMP levels in cells expressing the D_1 receptor with an EC_{50} of 7±3 nM. These results indicate potent agonist activity of l-stepholidine at the D_1 receptor. The effects on the D_2 receptor functional response were to block the dopamine-induced inhibition of forskolin-stimulated adenylyl cyclase activity in cells expressing the D_2 receptor (Fig. 1d).

D_1 and D_2 receptor occupancy

Only l-stepholidine, in comparison to haloperidol and clozapine, showed a dose-dependent striatal D_1 occupancy

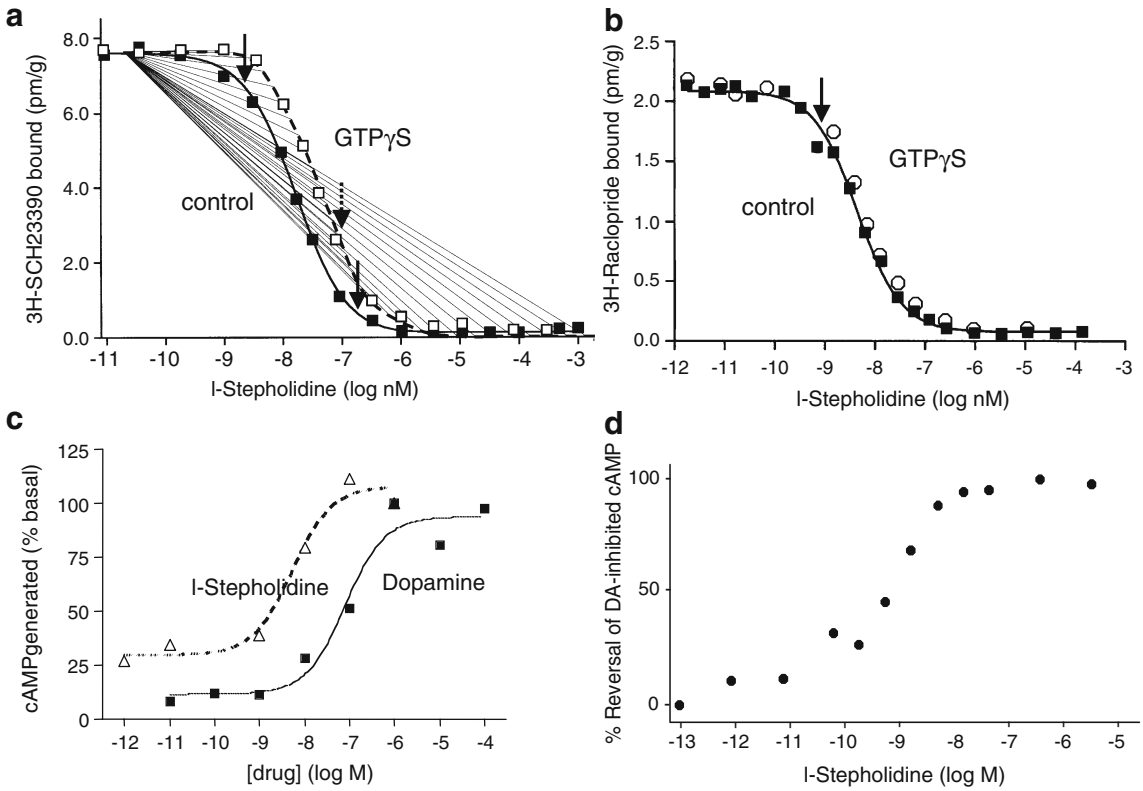

Fig. 1 Competition by l-stepholidine for **a** [^{3}H]SCH23390 or **b** [^{3}H] raclopride binding to membranes prepared from HEK cells expressing dopamine D_1 or D_2 receptors, respectively. The studies were performed in the absence (*solid symbols*) or presence (*clear symbols*) of GTPγS 100 μM. Each experiment was performed in triplicate and a representative experiment of n=3 experiments is shown. The *arrows* indicate the K_i values. Adenylyl cyclase activity was evaluated by the accumulation of cyclic AMP (cAMP) in cells stably expressing dopamine D_1 or D_2 receptors. In D_1 receptor expressing cells, the effects of **c** l-stepholidine and dopamine to stimulate cAMP production and in D_2 receptor expressing cells, the effect of **d** l-stepholidine to block dopamine (10 μM) inhibition of 1 μM forskolin-stimulated cAMP production. Each experiment was conducted in triplicate and shown are representative examples of n=3 or n=2 experiments

that exceeded 50% in the dose range tested, 1 h after administration (Fig. 2, Table 1). l-Stepholidine showed an ED_{50} of 8.08 mg/kg (CI 95% 5.08–11.08), while haloperidol (0.05–0.5 mg/kg) showed a maximal D_1RO of 23% and clozapine (0–40 mg/kg) a maximal D_1RO of 40%. All drugs showed dose-dependent striatal D_2 receptor occupancy (D_2RO) in the dose range tested, 1 h after drug administration (Fig. 2, Table 1). l-Stepholidine in a dose range of 1–30 mg/kg showed a D_2RO from 44% to 94%, while haloperidol in a dose range of 0.025–0.5 mg/kg showed a D_2RO from 49% to 86% and clozapine in a dose range of 5–40 mg/kg showed D_2RO from 29% to 60%.

Amphetamine and phencyclidine-induced locomotion

l-Stepholidine, haloperidol, and clozapine significantly inhibited amphetamine-induced locomotor activity in a dose-dependent manner (Fig. 3a, Table 1). The ED_{50} value

Table 1 Dopamine receptor occupancy and antipsychotic efficacy in animal models

Drug	D_2RO ED_{50} mg/kg (60 min)	D_1/D_2 RO ratio at highest tested dose	Catalepsy ED_{50} mg/kg (50 min)	AIL ED_{50} mg/kg (90 min)	PIL ED_{50} mg/kg (90 min)	CAR ED_{50} mg/kg (90 min)
l-Stepholidine	0.95	0.85	3.6	2.36	6.52	0.27[a]
Haloperidol	0.02	0.26	0.28	0.03	0.1	0.03
Clozapine	14.44	0.66	N.D.	4.27	20.96	7.77

N.D. Not determined
[a] Determined at 20 min post-treatment

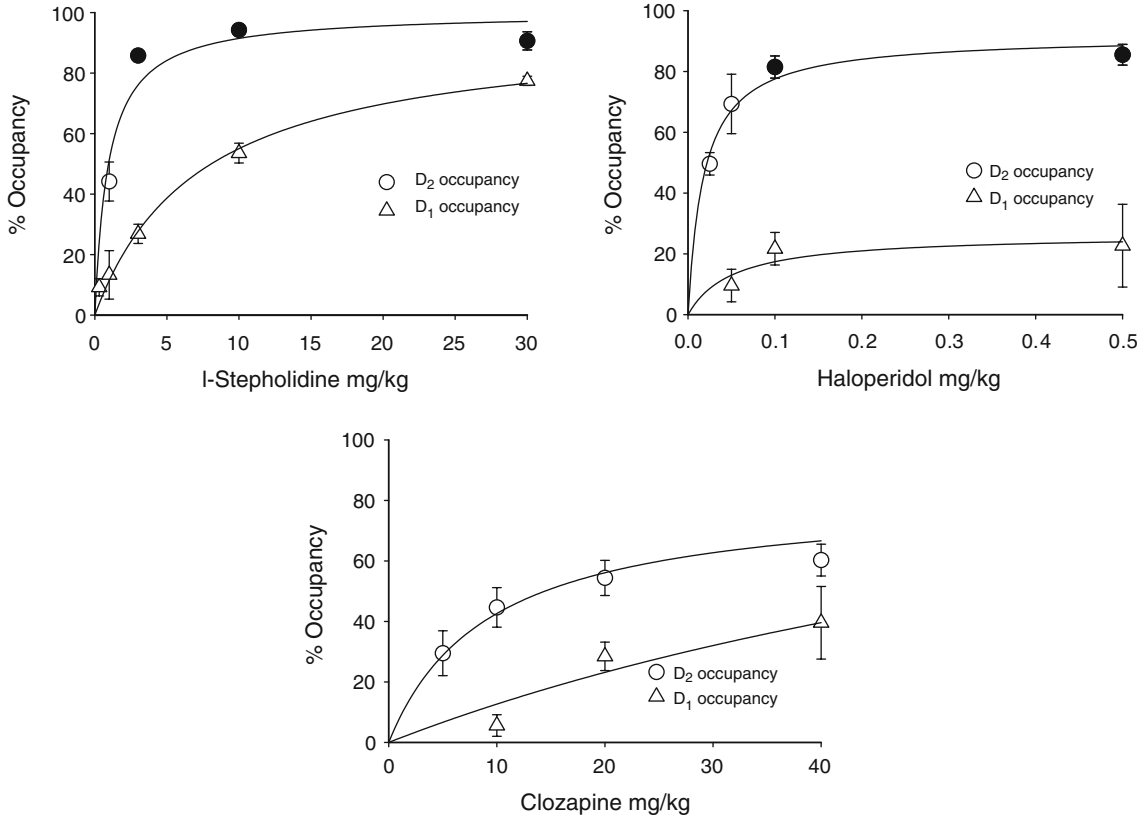

Fig. 2 Striatal $D_{1/2}$ receptor occupancy 1 h after administration of l-stepholidine, haloperidol, or clozapine (n=5 for each dose level). Values are expressed as mean ± SEM. *Filled symbols* in the D_2 receptor occupancy graph represent doses when animals started to show catalepsy. The curves were generated using a non-linear regression equation representing a rectangular hyperbola [$y=ax/(b+x)$] using Sigma Plot® software

for l-stepholidine was 2.36 (CI 95% 1.76–2.96), that of haloperidol 0.03 mg/kg (CI 95% 0.026–0.034), and that of clozapine 4.27 mg/kg (CI 95% 1.09–7.45). Similarly, all the three drugs reduced phencyclidine-induced locomotor activity in a dose-dependent manner (Fig. 3b, Table 1). The ED_{50} value for l-stepholidine was determined to be 6.52 mg/kg (CI 95% 5.68–7.36), that of haloperidol 0.1 mg/kg (CI 95% 0.02–0.18), and that of clozapine 20.96 (20.92–21).

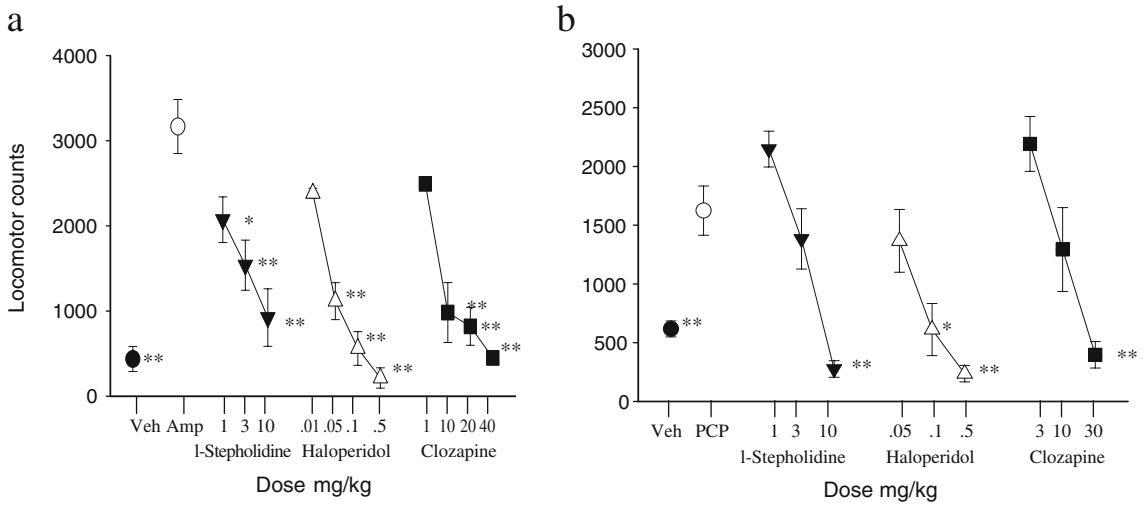

Fig. 3 The effects of l-stepholidine, haloperidol, and clozapine for each dose (n=5) on locomotor activity measured for 1-h duration after amphetamine (**a**) or phencyclidine (**b**) administration and expressed as mean ± SEM. The drugs or vehicle was administered 30 min prior to amphetamine or phencyclidine challenge. **p<0.001, *p<0.05, one-way ANOVA $F(12, 52)$=16.68; post hoc Dunnett (two-sided) with respect to pooled amphetamine treatment and **p<0.001, *p<0.05, one-way ANOVA $F(10, 64)$=8.87; post hoc Dunnett (two-sided) with respect to pooled phencyclidine treatment

Conditioned avoidance response

All drugs inhibited CAR significantly at the tested doses (Fig. 4, Table 1). l-Stepholidine was effective only at the 20-min time point and its ED_{50} 20 min post-treatment was 0.27 mg/kg (CI 95% −0.37–0.55). The remarkable aspect of the l-stepholidine effect was its rapid offset, with the animal showing normal CAR by 90 min after drug administration. Haloperidol showed a significant reduction in CAR 20 min after drug administration and its ED_{50} 90 min post-treatment was 0.03 mg/kg (CI 95% 0.004–0.06). Clozapine treatment also resulted in a significant reduction in CAR 20 min after drug administration and its ED_{50} (90 min) was 7.77 mg/kg (95%CI: 2.34–15.11). All drug-treated animals returned to their baseline 24 h after drug administration.

Catalepsy

l-Stepholidine and haloperidol induced catalepsy at higher doses (Table 1). In the case of l-stepholidine, one out of five animals at 3 mg/kg showed catalepsy, while all five at 10 mg/kg showed catalepsy. The ED_{50} value was 3.6 mg/kg. In the case of haloperidol, doses ≥0.1 mg/kg induced CAT, 1 h after drug treatment. The ED_{50} value was 0.28 mg/kg. None of the clozapine-treated animals showed CAT.

Fos expression

Induction of Fos in rats was measured over different doses in the nucleus accumbens as well as the dorsolateral striatum for all the three drugs (Fig. 5). l-Stepholidine significantly induced Fos in the nucleus accumbens (shell) at 3 mg/kg (Fig. 4). For haloperidol, significant Fos induction in the nucleus accumbens (shell) was demonstrated at 0.05 mg/kg. The lowest dose of clozapine that was tested, 7.5 mg/kg, led to significant Fos induction in the nucleus accumbens (shell).

l-Stepholidine at 10 mg/kg induced CAT and Fos in the dorsolateral striatum, and these two measures were correlated (Fig. 6). Haloperidol at 0.5 mg/kg, a dose that has a

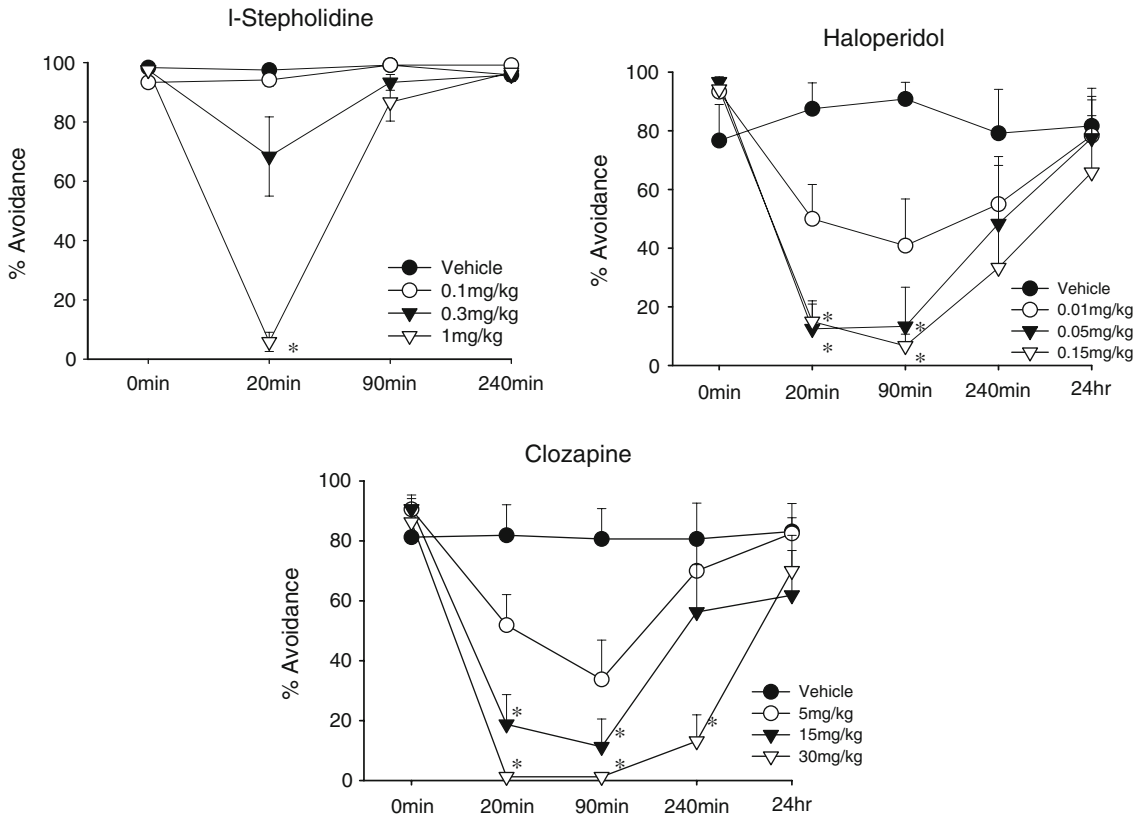

Fig. 4 Effect of l-stepholidine (n=6), haloperidol (n=6), and clozapine (n=6) on the performance of conditioned avoidance response in rats after single subcutaneous injection. The animals served as their own controls using a within-subject design. Values of percentage inhibition of avoidance are expressed as mean ± SEM. The avoidance values were analyzed for each drug in a repeated measures analysis of variance with dose (vehicle, three drug doses) as a within-subject factor for each time point separately. If the sphericity assumption was not met, the Huynh–Feldt correction was applied, and the main effect of dose was significant at least at one time point for all the drugs. Post hoc comparisons were performed using the Bonferroni adjustment for multiple comparisons and the level of significance indicated in the figure is that with respect to vehicle treatment (*p<0.05)

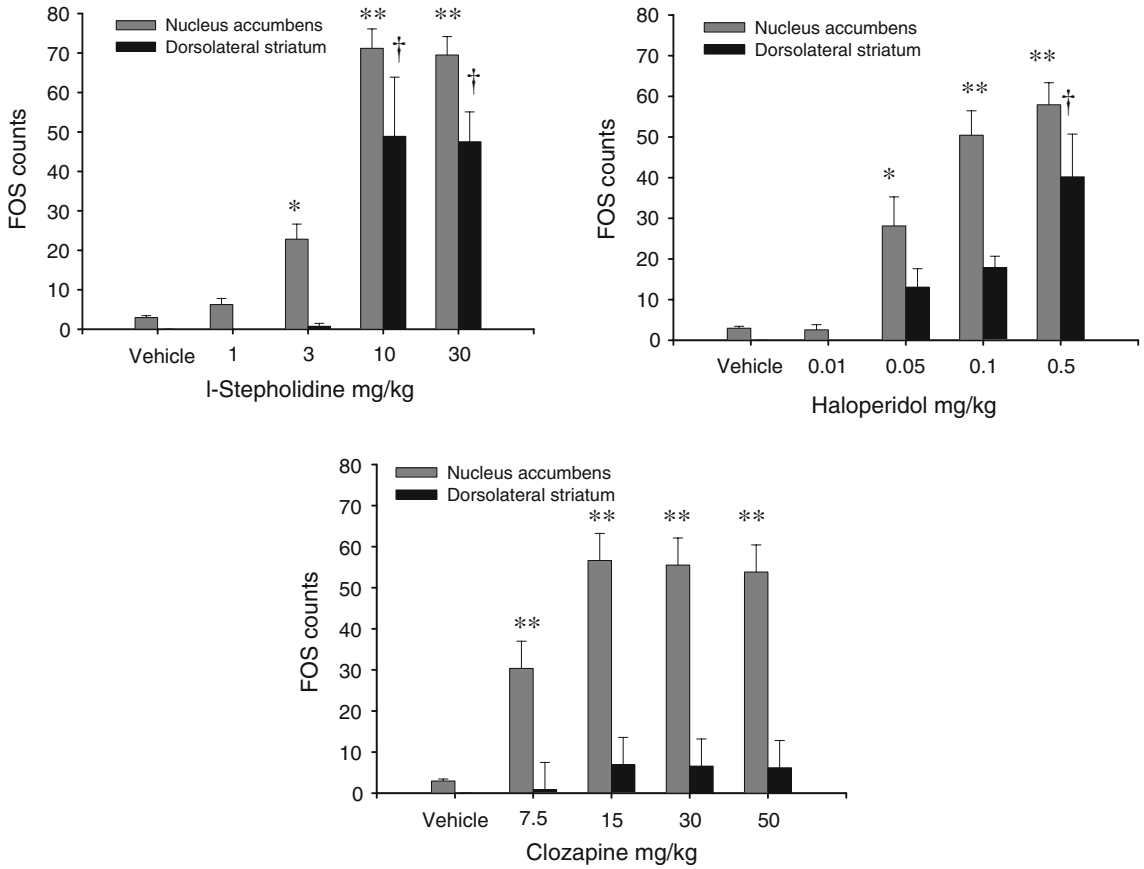

Fig. 5 Fos expression in the nucleus accumbens and dorsolateral striatum due to drug treatment (n=4 for each dose). Rats were killed 2 h after drug administration and Fos immunoreactive nuclei expressed as mean ± SEM were counted within a 400×400-μm grid in the specified brain regions. $^{**}p<0.001$, $^{*}p<0.05$, one-way ANOVA $F(12, 47)=29.56$; post hoc Dunnett (two-sided) with respect to pooled vehicle control of nucleus accumbens. †$p<0.001$, one-way ANOVA $F(12, 47)=13.56$; post hoc Dunnett (two-sided) with respect to pooled vehicle treatment of dorsolateral striatum

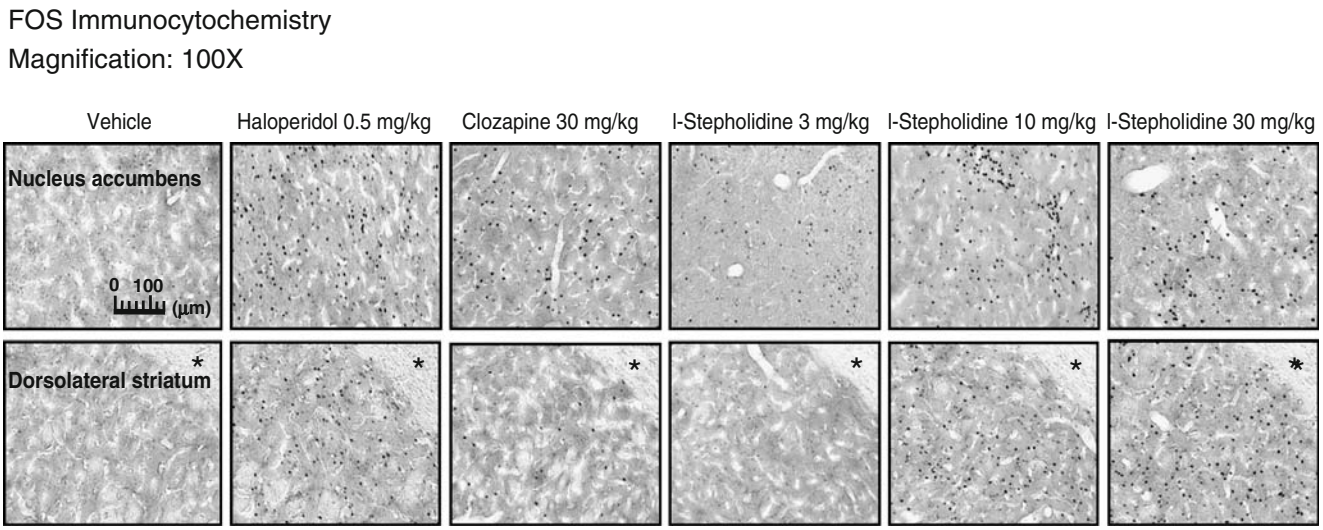

Fig. 6 Effects of acute treatment with haloperidol, clozapine, or l-stepholidine on Fos induction in the nucleus accumbens (shell) and dorsolateral striatum in rats. *Asterisks* indicate the dorsolateral corpus callosum

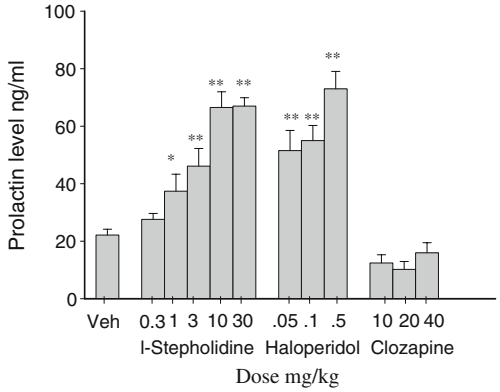

Fig. 7 Plasma prolactin levels after l-stepholidine, haloperidol, and clozapine treatment measured from plasma samples obtained from the occupancy experiment 1 h after subcutaneous administration (minimum of n=4 for each dose). Values are expressed as mean ± SEM, *p<0.05, **p<0.001, one-way ANOVA $F(11, 57)=28.54$; post hoc Dunnett (two-sided) with respect to the pooled vehicle control

propensity for inducing CAT, clearly induced high levels of Fos. Clozapine in the dose range tested did not significantly induce Fos in the dorsolateral striatum and also did not produce CAT.

Prolactin

l-Stepholidine caused a dose-dependent increase in plasma prolactin levels and so did haloperidol, while clozapine treatment did not lead to an increase in prolactin levels, 1 h after drug administration at the doses tested (Fig. 7).

D_2 receptor knockout mice—locomotor activity and Fos immunohistochemistry

In order to establish a dose for testing l-stepholidine in the D_2 knockout mice, l-stepholidine was evaluated for catalepsy in the background strain (C57/BL6) of the knockout mice. Catalepsy was noticed 30 min after drug administration in the 5 and 10 mg/kg groups and lasted up to 1 h in the wild-type mice. ED_{50} at the 1 h time point was 4.7 mg/kg and hence 6 mg/kg was chosen for all studies in the D_2 knockout mice, based on the rat data whereby equivalent doses would have achieved high D_2/D_1 receptor occupancies. In the locomotor study, l-stepholidine (6 mg/kg) failed to enhance locomotor activity compared to vehicle-treated animals, while SKF81297 (10 mg/kg), a positive control, induced robust locomotor activity over a 120-min period (Fig. 8). In the Fos experiment, l-stepholidine as well as SKF81297 induced significant Fos expression in the prefrontal cortex and nucleus accumbens (Fig. 9). Also, Fos expression due to l-stepholidine was reversed by pretreatment with the D_1 antagonist SKF83566 (Figs. 9 and 10).

Time course receptor occupancy and single dose pharmacokinetics

l-Stepholidine had a significant effect only at the 20-min time point in the CAR assay, and a separate study to look at the time course of D_2RO occupancy of l-stepholidine (3 mg/kg) revealed a sudden drop off in D_2 receptor

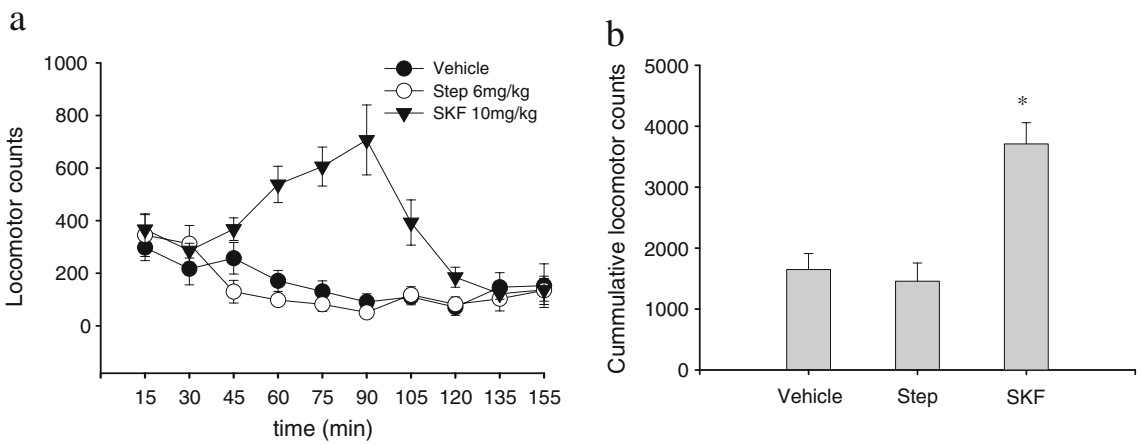

Fig. 8 Effect of l-stepholidine (*Step*) and SKF81297 (*SKF*) on locomotor activity in D_2KO mice (n=7) after single subcutaneous injection. The animals served as their own controls using a within-subject design. **a** Values of locomotor counts are expressed as mean ± SEM for the entire duration in bins of 15 min after drug/vehicle administration. **b** The cumulative values were analyzed for each drug in a repeated measures analysis of variance with dose (vehicle, three drug doses) as a within-subject factor for each time segment separately. If the sphericity assumption was not met, the Huynh–Feldt correction was applied and the main effect of dose was significant at least at one time point for all the drugs. Post hoc comparisons were performed using the Bonferroni adjustment for multiple comparisons and the level of significance indicated in the figure is that with respect to vehicle treatment (*p<0.05)

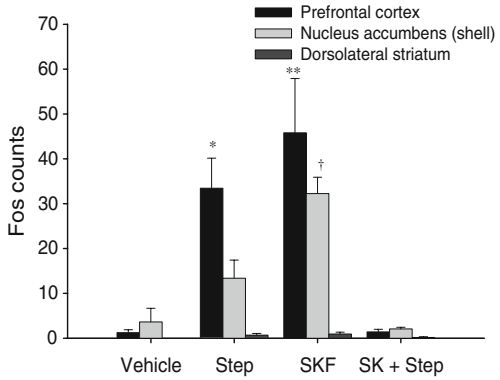

Fig. 9 Fos expression in the prefrontal cortex, nucleus accumbens (shell) and dorsolateral striatum due to drug treatment [Vehicle (n=4), l-stepholidine 6 mg/kg (Step; n=4), SKF81297 10 mg/kg (SKF; n=4), SKF83566 0.5 mg/kg (SK) + Step 6 mg/kg (n=3)] in D2KO mice. Mice were killed 2 h after drug administration and Fos immunoreactive nuclei expressed as mean ± SEM was counted within a 270×270-μm grid in the specified brain regions. $^{**}p<0.005$, $^{*}p<0.05$, one-way ANOVA $F(3, 11)=9.22$; post hoc Dunnett (two-sided) with respect vehicle control of prefrontal cortex. †$p<0.05$ one-way ANOVA $F(3, 11)=17.14$; post hoc Dunnett (two-sided) with respect to vehicle control of nucleus accumbens (shell)

occupancy by 4 h in contrast to haloperidol (0.5 mg/kg) and clozapine (20 mg/kg) which showed more sustained occupancies and more sustained CAR effects (Table 2). This prompted us to examine its pharmacokinetic properties in detail. A sensitive method for the quantification of l-stepholidine was used. The results are illustrated in Fig. 11, and preliminary pharmacokinetics using non-compartmental analysis has been tabulated in Table 3. The results point to a rapid distribution and elimination from plasma as well as brain regions. The $AUC_{\text{brain tissue/plasma}}$ ratio (<1) indicates poor brain penetrability. Also, the MTT of l-stepholidine through the system (less than an hour) is consistent with the pharmacodynamic effects on CAR by this drug.

Discussion

l-Stepholidine showed a dose-dependent striatal D_2RO and in comparison to haloperidol and clozapine, its ED_{50} value for D_2RO followed an order comparable to its in vitro affinity (haloperiodol > l-stepholidine > clozapine), consistent with its actions on AIL, CAR, and prolactin levels. The results indicate a preference for antipsychotic-like activity versus motor side effects and also preferential Fos expression in the nucleus accumbens over dorsolateral striatum. Its effects on PIL are encouraging, as this is largely viewed as a model reflecting negative symptomology (Jentsh and Roth 1999). While it did show unequivocal D_1 agonism in vitro, it showed only limited D_1RO, and its functional effects, while indicative of D_1 agonism in vivo, were weak. In the light of these findings (including in vitro results from the broad screen), we focus our discussion on the most critical contributions of this report—evidence for the D_1 receptor action of l-stepholidine, its comparison to other antipsychotics, the implications of its fast kinetics, and the potential for the compound to enhance cognitive deficits.

The nature of D_1 receptor agonism due to l-stepholidine has been in the past characterized as that of a partial to a full agonist (Dong et al. 1997b; Zou et al. 1997). In normal animals, D_1 agonism exhibited by l-stepholidine is reportedly weak and becomes functionally more efficacious in dopamine-depleted states (e.g., after a 6-OHDA striatal lesion, Zou et al. 1997; Liu et al. 1999). Our radioligand binding studies showed that l-stepholidine recognized two affinity states of the D_1 receptor, and the sensitivity of the high affinity state to a GTP analogue indicated that it behaved as an agonist, with higher affinity for the G protein-associated D_1 receptor. In contrast, l-stepholidine recognized only a single affinity state of the D_2 receptor that showed no shift in affinity with the GTP analogue, as typically seen with a receptor antagonist which is unable to discriminate between the different receptor forms. The effects of l-stepholidine on the functional responses of D_1 and D_2

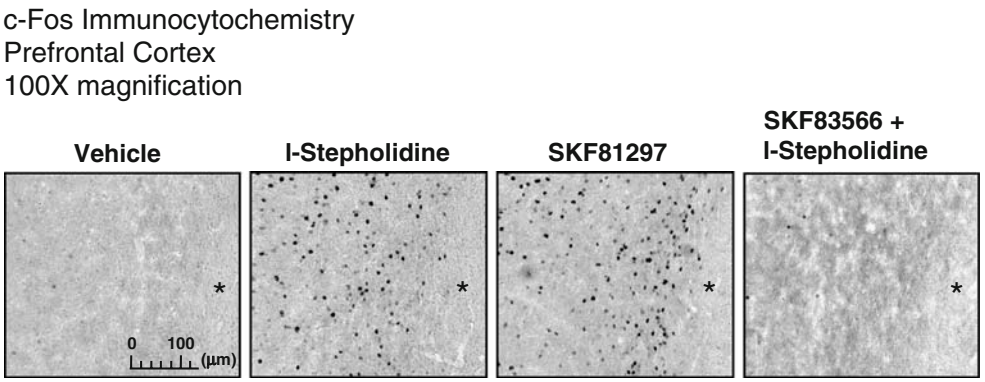

Fig. 10 Effects of acute treatment with l-stepholidine, SKF81297, and SKF83566 + l-stepholidine on Fos induction in the prelimbic cortex in D_2KO mice. *Asterisks* indicate the corpus callosum

Table 2 Time course of percentage D_2 striatal occupancy of l-stepholidine, haloperidol, and clozapine

Drug	1 h	2 h	4 h	6 h	8 h	24 h
l-Stepholidine, 3 mg/kg	85.8±0.23[a]	71.44±2.88	<5%	N.D.	N.D.	N.D.
Haloperidol, 0.5 mg/kg	85.5±3.4[a]	90.46±2.24[a]	87.92±0.97[a]	N.D.	79.73±0.9[a]	36.93±5.94
Clozapine, 20 mg/kg	54.38±5.81	61.73±1.74	33.7±6.86	24.53±6.7	N.D.	N.D.

Values are mean ± SEM obtaining using at least four rats at each dose and time point.
N.D. Not determined
[a] Catalepsy present

receptors, as indicated by the effects on adenylyl cyclase activity in vitro, showed D_1 agonism and D_2 antagonism. In vivo, the dual actions have been corroborated in several earlier studies (review by Jin et al. 2002); however, it is not clear if these actions are exhibited in clinically relevant doses.

In an effort to evaluate D_1 receptor effects in vivo, we extended our study by evaluating D_1 agonism in D_2KO mice where the lack of D_2 receptors would help elucidate D_1 agonistic actions of l-stepholidine. The dose of l-stepholidine evaluated in the D_2KO mice experiments was decided based on the dose needed to induce catalepsy in the wild-type background strain of the D_2KO mice, based on the rat occupancy results that this dose would induce nearly 80% D_2RO. In stark contrast to the robust locomotor response observed with SKF81297, l-stepholidine did not induce locomotor activity in D_2KO mice. Phenotypic resolution of several behaviors (locomotor, habituation, grooming, orofacial movements, etc.) that have a very complex nature of interaction between dopaminergic receptors is ongoing, and the action of l-stepholidine needs to be further evaluated in this context (McNamara et al. 2002; O'Sullivan et al. 2005; O'Sullivan et al. 2006). D_1 receptor agonists are known to have varying abilities to activate adenylyl cyclase or phosphoinositide (PI) hydrolysis, and there is limited association between in vitro effectiveness at stimulating adenylyl cyclase by D_1 agonists (or PI hydrolysis, another second messenger associated with D_1 activity) and behavioral effects that they exhibit (Arnt et al. 1992; Terry and Katz 1992; Desai et al. 2005; Ryman-Rasmussen et al. 2005). Also, an emerging concept of functional selectivity (differential activation of signaling pathways mediated via a single G protein-coupled receptor) or the formation of unique receptor complexes could perhaps account for these outcomes (Rashid et al. 2007; Urban et al. 2007).

As it was not clear if D_1 agonism was exhibited in the behavioral model in D_2KO mice, we investigated l-stepholidine's ability to induce immediate early genes in the same D_2KO mice strain. Interestingly, l-stepholidine induced Fos in the prelimbic area of the prefrontal cortex as well as nucleus accumbens (shell) and the D_1 antagonist SKF83566 blocked this Fos expression. The D_1 agonist SKF 81297 which also induced Fos in a similar fashion as l-stepholidine, contrasted itself from l-stepholidine in its locomotor actions. The results of the Fos expression study in D_2KO mice clearly prove that D_1 agonism is exhibited by l-

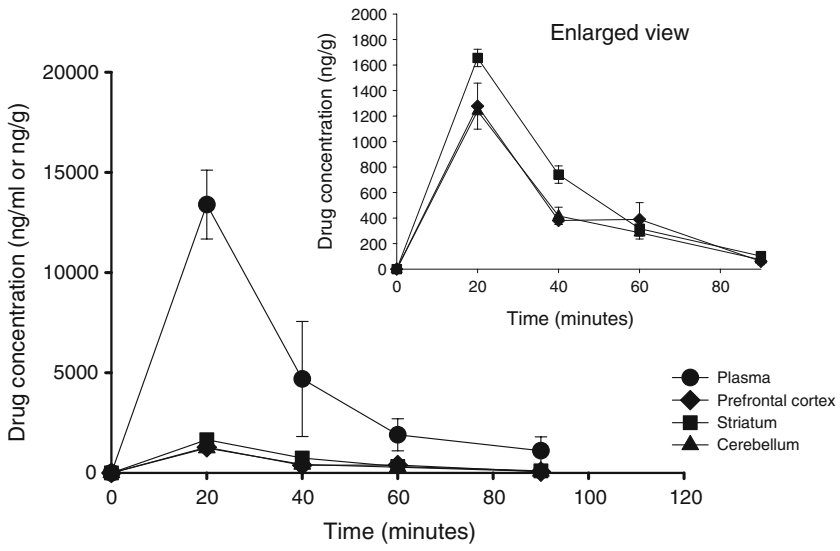

Fig. 11 l-Stepholidine in plasma and brain regions following subcutaneous (10 mg/kg) administration to rats. Values of drug levels (nanogram per milliliter or nanogram per gram) expressed as mean ± SEM of five rats at each time point are taken from Odontiadis et al. (2007)

Table 3 Non-compartmental pharmacokinetic parameters following subcutaneous administration of l-stepholidine (10 mg/kg)

Compartment	$AUC_{(0-tlast)}$ (ng h/ml or g)	$AUC_{(0-tlast)}$ ratio Brain tissue/plasma	$AUC_{(0-\infty)}$ (ng h/ml or g)	$AUC_{(0-\infty)}$ ratio Brain tissue/plasma	$AUMC_{(0-\infty)}$ ($ng.h^2$/ml or g)	MTT (h)
Plasma	7,066	–	7,746	–	5,145	0.66
Prefrontal cortex	729	0.1	763	0.1	490	0.64
Striatum	954	0.14	1,011	0.13	650	0.64
Cerebellum	687	0.1	728	0.09	376	0.52

Values are mean ± SEM obtained using five rats at each time point.

stepholidine in a dose range that overlaps with its D_2 antagonist actions (emergence of catalepsy) in the normal background strain of the knockout mice. This is important since it would predict that functional D_1 agonism would be exhibited at doses resulting in the expected clinically effective D_2RO range of 60–85%, although the effect could be weak.

Comparisons with other antipsychotics have not been perfect as the results of l-stepholidine in these assays could have been affected by two factors—concomitant D_1 agonism and its pharmacokinetic profile. As D_1 agonism seems to be weak, the focus shifted to its pharmacokinetic profile. The time course of D_2RO as well as the single dose pharmacokinetics explains the very short duration of action in CAR and very high doses needed to inhibit AIL/PIL. The brain–plasma ratio ($AUC_{brain/plasma}$) of stepholidine indicates less penetrability to the brain similar to risperidone (<1), while for most CNS active drugs including haloperidol, olanzapine, and clozapine, the brain concentrations are several fold higher compared to plasma levels (Aravagiri et al. 1999; Aravagiri and Marder 2002; Doran et al. 2005). The MTT, a non-compartmental measure of the average time drug molecules spend in the system, indicates rapid removal of l-stepholidine from the body. This poses a challenge to maintain sustained threshold doses of the drug for antipsychotic activity and perhaps explains the effects in CAR.

The present study lays a foundation for further clinical investigations, and doses determined based on D_2RO can be translated to human studies using [^{11}C]raclopride positron emission tomography. Our preclinical findings support earlier studies carried out by Jin's group, provide direct documentation of in vivo binding to both D_1 and D_2 receptors, shed more light on the question of D_1 agonism, and present a comparative picture of its antipsychotic efficacy in animal models supported by a brief pharmacokinetic assessment. One of the major limitations of this series of experiments is that the animal models used in the present study mostly reflect efficacy against positive symptoms in schizophrenia, and we did not test the claim of potential cognitive enhancement directly. With the emergence of improved animal models for cognitive function, l-stepholidine's putative precognitive benefits can be studied more directly in future studies (Floresco et al. 2005). Studies both at the preclinical and clinical levels have to be designed carefully as D_1 receptor activity follows the "inverted-U" dose–response where too little or too much D_1 stimulation could impair working memory (Robbins 2005) and chronic treatment could lead to downregulation. Intermittent patterns of administration could overcome these problems (Castner et al. 2000). Also preclinical paradigms should consider the fact that acute administration could affect locomotor activity due to D_2 antagonism and false negatives could result as most cognitive tasks in animals involve some degree of motor activity.

On acute systemic administration, l-stepholidine at low dose increase dopamine levels in the nucleus accumbens as well as in the striatum, a characteristic action of acute presynaptic D_2 receptor blockade (Huang et al. 1991; Chen et al. 1992). Also local administration of l-stepholidine into the medial prefrontal cortex has resulted in reduced dopamine levels in the nucleus accumbens possibly mediated by D_1 receptor agonism, although the exact mechanism is still not clear (Zhu et al. 2000; Olsen and Duvauchelle 2001). These actions have the potential to stabilize the dopaminergic system. Also, recent findings that l-stepholidine can potently enhance synaptically evoked NMDA receptor-mediated post-synaptic excitatory currents in prefrontal cortical neurons by its actions on D_1 receptors are encouraging as this mechanism is postulated to be hypofunctional in schizophrenic subjects, leading to cognitive disability (Yang and Chen 2005). Whether this translates into clinically meaningful precognitive benefits remains to be studied in future clinical studies, but clearly prefrontal hypofunctionality has been implicated in specific deficits in attentional control and working memory in schizophrenia, and appropriate modulation via D_1 receptors is thought to have beneficial outcomes (Robbins 2005). Furthermore, the finding that l-stepholidine promotes neurogenesis (Guo et al. 2002) and protects against cortical neuronal neurotoxicity could theoretically translate in better long-term outcomes in schizophrenia (Zhang et al. 2005). In summary, the preclinical assessment of l-stepholidine as an antipsychotic and its side effect profile seems promising to target the positive and negative symptoms of schizo-

phrenia, and it will in all likelihood be an excellent addition to the therapeutically useful atypical antipsychotic drugs.

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References

Abi-Dargham A (2004) Do we still believe in the dopamine hypothesis? New data bring new evidence. Int J Neuropsychopharmacol 7(Suppl 1):S1–5

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M (2002) Prefrontal dopamine D_1 receptors and working memory in schizophrenia. J Neurosci 22:3708–3719

Aravagiri M, Marder SR (2002) Brain, plasma and tissue pharmacokinetics of risperidone and 9-hydroxyrisperidone after separate oral administration to rats. Psychopharmacology (Berl) 159:424–431

Aravagiri M, Teper Y, Marder SR (1999) Pharmacokinetics and tissue distribution of olanzapine in rats. Biopharm Drug Dispos 20:369–377

Arnt J, Skarsfeldt T (1998) Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. Neuropsychopharmacology 18:63–101

Arnt J, Hyttel J, Sanchez C (1992) Partial and full dopamine D_1 receptor agonists in mice and rats: relation between behavioural effects and stimulation of adenylate cyclase activity in vitro. Eur J Pharmacol 213:259–267

Castner SA, Williams GV, Goldman-Rakic PS (2000) Reversal of antipsychotic-induced working memory deficits by short-term dopamine D1 receptor stimulation. Science 287:2020–2022

Chen LJ, Guo X, Wang QM, Jin GZ (1992) Feed-back regulation of presynaptic D2 receptors blockaded by l-stepholidine and l-tetrahydropalmatine. Acta Pharmacol Sin 13:442–445

Desai RI, Terry P, Katz JL (2005) A comparison of the locomotor stimulant effects of D_1-like receptor agonists in mice. Pharmacol Biochem Behav 81:843–848

Dong ZJ, Chen LJ, Jin GZ, Creese I (1997a) GTP regulation of (−)-stepholidine binding to R(H) of D_1 dopamine receptors in calf striatum. Biochem Pharmacol 54:227–232

Dong ZJ, Guo X, Chen LJ, Han YF, Jin GZ (1997b) Dual actions of (−)-stepholidine on the dopamine receptor-mediated adenylate cyclase activity in rat corpus striatum. Life Sci 61:465–472

Doran A, Obach RS, Smith BJ, Hosea NA, Becker S, Callegari E, Chen C, Chen X, Choo E, Cianfrogna J, Cox LM, Gibbs JP, Gibbs MA, Hatch H, Hop CE, Kasman IN, Laperle J, Liu J, Liu X, Logman M, Maclin D, Nedza FM, Nelson F, Olson E, Rahematpura S, Raunig D, Rogers S, Schmidt K, Spracklin DK, Szewc M, Troutman M, Tseng E, Tu M, Van Deusen JW, Venkatakrishnan K, Walens G, Wang EQ, Wong D, Yasgar AS, Zhang C (2005) The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. Drug Metab Dispos 33:165–174

Ellenbroek BA, Zhang XX, Jin GZ (2006) Effects of (−)stepholidine in animal models for schizophrenia. Acta Pharmacol Sin 27:1111–1118

Finney DJ (1971) Probit analysis. Cambridge University Press, London

Floresco SB, Geyer MA, Gold LH, Grace AA (2005) Developing predictive animal models and establishing a preclinical trials network for assessing treatment effects on cognition in schizophrenia. Schizophr Bull 31:888–894

Franklin KBJ, Paxinos G (1997) The mouse brain in stereotaxic coordinates. Academic, San Diego

Goldman-Rakic PS, Muly EC 3rd, Williams GV (2000) D_1 receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 31:295–301

Guo H, Yu Y, Xing L, Jin GZ, Zhou J (2002) (−)-Stepholidine promotes proliferation and neuronal differentiation of rat embryonic striatal precursor cells in vitro. Neuroreport 13:2085–2089

Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L (1994) Distribution of D_1- and D_2-dopamine receptors, and dopamine and its metabolites in the human brain. Neuropsychopharmacology 11:245–256

Huang KX, Sun BC, Gonon FG, Jin GZ (1991) Effects of tetrahydroprotoberberines on dopamine release and 3,4-dihydroxyphenylacetic acid level in corpus striatum measured by in vivo voltammetry. Acta Pharmacol Sin 12:32–36

Jentsh JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20:201–225

Jin GZ (1987) (−)-Tetrahydropalmatine and its analogues as new dopamine receptor antagonists. Trends Pharmacol Sci 8:81–82

Jin GZ, Sun BC (1995) Neuropharmacological effects of (−)-stepholidine and its analogues on brain dopaminergic system. Adv Exp Med Biol 363:27–28

Jin GZ, Huang KX, Sun BC (1992) Dual actions of (−)-stepholidine on dopamine receptor subtypes after substantia nigra lesion. Neurochem Int 20(Suppl):175S–178S

Jin GZ, Zhu ZT, Fu Y (2002) (−)-Stepholidine: a potential novel antipsychotic drug with dual D_1 receptor agonist and D_2 receptor antagonist actions. Trends Pharmacol Sci 23:4–7

Kane JM, Malhotra A (2003) The future of pharmacotherapy for schizophrenia. World Psychiatry 2:81–86

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. J Pharmacol Exp Ther 305:625–631

Lidow MS, Goldman-Rakic PS, Gallager DW, Rakic P (1991) Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [^{3}H] raclopride, [^{3}H]spiperone and [^{3}H]SCH23390. Neuroscience 40:657–671

Liu J, Guo X, Wang BC, Jin GZ (1999) Increased phosphorylation of DARPP-32 by D1 agonistic action of l-stepholidine in the 6-OHDA-lesioned rat striatum. Acta Physiologica Sinica 51:65–72

McNamara FN, Clifford JJ, Tighe O, Kinsella A, Drago J, Fuchs S, Croke DT, Waddington JL (2002) Phenotypic, ethologically based resolution of spontaneous and D_2-like vs D_1-like agonist-induced behavioural topography in mice with congenic D(3) dopamine receptor "knockout". Synapse 46:19–31

Mo YQ, Jin XL, Chen YT, Jin GZ, Shi WX (2005) Effects of l-stepholidine on forebrain Fos expression: comparison with clozapine and haloperidol. Neuropsychopharmacology 30:261–267

Natesan S, Reckless GE, Nobrega JN, Fletcher PJ, Kapur S (2006) Dissociation between in vivo occupancy and functional antagonism of dopamine D(2) receptors: comparing aripiprazole to other antipsychotics in animal models. Neuropsychopharmacology 31:1854–1863

Odontiadis J, Mackenzie EM, Natesan S, Mamo D, Kapur S, Baker GB (2007) Quantification of l-stepholidine in rat brain and plasma by high performance liquid chromatography combined with fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci 850:544–547

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M (1997) Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. Nature 385:634–636

Olsen CM, Duvauchelle CL (2001) Intra-prefrontal cortex injections of SCH 23390 influence nucleus accumbens dopamine levels 24 h post-infusion. Brain Res 922:80–86

O'Sullivan GJ, Kinsella A, Sibley DR, Tighe O, Croke DT, Waddington JL (2005) Ethological resolution of behavioural topography and D_1-like versus D_2-like agonist responses in congenic D_5 dopamine receptor mutants: identification of D_5:D_2-like interactions. Synapse 55:201–211

O'Sullivan GJ, Kinsella A, Grandy DK, Tighe O, Croke DT, Waddington JL (2006) Ethological resolution of behavioral topography and D_2-like vs. D_1-like agonist responses in congenic D_4 dopamine receptor "knockouts": identification of D_4:D_1-like interactions. Synapse 59:107–118

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic, New York

Rashid AJ, So CH, Kong MM, Furtak T, El-Ghundi M, Cheng R, O'Dowd BF, George SR (2007) D_1–D_2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci U S A 104:654–659

Robbins TW (2005) Chemistry of the mind: neurochemical modulation of prefrontal cortical function. J Comp Neurol 493:140–146

Robertson GS, Matsumura H, Fibiger HC (1994) Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. J Pharmacol Exp Ther 271:1058–1066

vhRyman-Rasmussen JP, Nichols DE, Mailman RB (2005) Differential activation of adenylate cyclase and receptor internalization by novel dopamine D_1 receptor agonists. Mol Pharmacol 68:1039–1048

So CH, Varghese G, Curley KJ, Kong MM, Alijaniaram M, Ji X, Nguyen T, O'Dowd BF, George SR (2005) D_1 and D_2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. Mol Pharmacol 68:568–578

Terry P, Katz JL (1992) Differential antagonism of the effects of dopamine D_1-receptor agonists on feeding behavior in the rat. Psychopharmacology (Berl) 109:403–409

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H, Javitch JA, Roth BL, Christopoulos A, Sexton PM, Miller KJ, Spedding M, Mailman RB (2007) Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320:1–13

Wadenberg ML, Kapur S, Soliman A, Jones C, Vaccarino F (2000) Dopamine D_2 receptor occupancy predicts catalepsy and the suppression of conditioned avoidance response behavior in rats. Psychopharmacology (Berl) 150:422–429

Yamaoka K, Nakagawa T, Uno T (1978) Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm 6:547–558

Yang CR, Chen L (2005) Targeting prefrontal cortical dopamine D_1 and *N*-methyl-D-aspartate receptor interactions in schizophrenia treatment. Neuroscientist 11:452–470

Zhang ZD, Zhou CM, Jin GZ, Zhang X, Yang L (1990) Pharmacokinetics and autoradiography of [^{3}H] or [^{14}C]stepholidine. Acta Pharmacol Sin 11:289–292

Zhang X, Sun BC, Jin GZ (1997) Atypical neuroleptic properties of l-stepholidine. Science in China (Series C) 40:532–538

Zhang L, Zhou R, Xiang G (2005) Stepholidine protects against H_2O_2 neurotoxicity in rat cortical neurons by activation of Akt. Neurosci Lett 383:328–332

Zhu ZT, Wu WR, Fu Y, Jin GZ (2000) I-stepholidine facilitates inhibition of mPFC DA receptors on subcortical NAc DA release. Acta Pharmacol Sin 21:663–667

Zou LL, Chen Y, Song YY, Jin GZ (1996) Effect of (−)-stepholidine on serum prolactin level of female rats. Acta Pharmacol Sin 17:311–314

Zou LL, Liu J, Jin GZ (1997) Involvement of receptor reserve in D_1 agonistic action of (−)-stepholidine in lesioned rats. Biochem Pharmacol 54:233–240