Expression of dopamine D_1 receptor in Sf9 insect cells and agonism of *l*-12-chloroscoulerine on recombinant D_1 receptor[1]

HE You, JIN Wen-Qiao[2], SHEN Qing-Xiang[3], CHEN Xin-Jian, JIN Guo-Zhang

Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031; [3]Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China

KEY WORDS recombinant proteins; dopamine D_1 receptors; insects; baculoviridae; *l*-12-chloroscoulerine; cyclic AMP

ABSTRACT

AIM: To express dopamine D_1 receptor in baculovirus-Sf9 cell system, and to investigate the effects of *l*-12-chloroscoulerine (*l*-CSL) on the recombinant D_1 receptor (D_1R). **METHODS:** The recombinant baculovirus, *Autographa californica* nuclear polyhedrosis virus bearing D_1R (AcNPV- D_1R) was generated, and then was used to produce recombinant D_1R in Sf9 insect cells. Expression of D_1R in Sf9 cells was monitored by [^{3}H]SCH23390 binding assay. The effects of *l*-CSL on recombinant D_1R were investigated by [^{3}H]SCH23390 binding assay and cAMP assay. **RESULTS:** The recombinant baculovirus AcNPV bearing D_1R cDNA was generated, and was successfully expressed in Sf9 insect cells. The expression level of (B_{max}) was (0.94±0.06) nmol/g protein. The K_d value of [^{3}H]SCH23390 was (1.9±0.3) nmol/L, which was consistent with the previous results from calf striutam tissues. *l*-CSL had a high affinity to recombinant D_1R with K_i value of (6.3±1.4) nmol/L, and increased the intracellular cAMP level in a concentration-dependent manner with EC_{50} value of 0.72 μmol/L and 95 % confidence limit was 0.67-0.77 μmol/L. Thus *l*-CSL has the D_1 receptor agonism. **CONCLUSION:** An efficient baculovirus-Sf9 insect cell system for dopamine D_1 receptor was constructed and *l*-CSL presented the D_1 receptor agonism on cellular-molecular level directly.

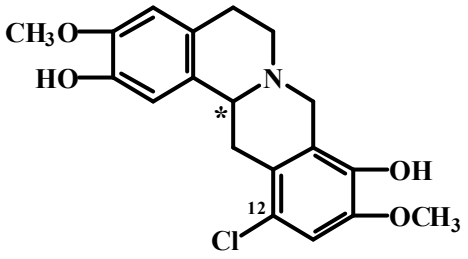
INTRODUCTION

Dopamine (DA) receptor, with seven transmembrane regions, is one of the members in G protein-coupled receptor family. Five DA receptor subtypes have been identified by molecular cloning, which are nominated D_1, D_2, D_3, D_4, and D_5. The D_1 and D_5 receptors are grouped into the pharmacological D_1-like receptor subtype, which activate the second message cAMP level via G_s protein in intracellular signal transduction. While D_2, D_3, and D_4 receptors belong to the pharmacological D_2-like receptor subtype, which decrease the second message cAMP level via $G_{i/o}$ protein.

12-Chloroscoulerine (CSL) is a synthetic compound of tetrahydroprotoberberines. Due to presence of one asymmetric carbon atom, the effects of CSL enantiomers were studied. The results showed that *l*-12-chloroscoulerine (*l*-CSL) is really more potent one in the CSL enantiomers[1-4]. In biochemical determination,

[1] Project supported by the National Natural Science Foundation of China (No 30070866) and Grant G (1998) 051126 from the Ministry of Science and Technology of China.
[2] Correspondence to Prof JIN Wen-Qiao.
Phn 86-21-6431-1833, ext 510. Fax 86-21-6437-0269.
E-mail wqjin@mail.shcnc.ac.cn
Received 2002-07-12 Accepted 2002-12-12

l-CSL has high affinities for both D_1 and D_2 receptors[4]. In the electrophysiological recording, *l*-CSL reversed the apomorphine (APO)-induced inhibition of firing activity on nigral DA neurons[2]. In the behavioral assay, *l*-CSL antagonized the APO-induced stereotype in rats and induced the catalepsy[4]. All these results indicate that *l*-CSL is a D_2 antagonist.



Chemical structure of 12-chloroscoulerine

In the 6-OHDA unilateral lesioned rats, however, *l*-CSL induced contralateral rotation[4], which was similar to the D_1-selective agonist SKF38393. It has reported that the D_1 receptors were supersensitive in the 6-OHDA unilateral lesioned rats[5-7]. The results suggested that *l*-CSL showed the D_1 agonism in the lesioned rats.

To clarify the D_1 agonism of *l*-CSL, the recombinant D_1 receptor was expressed in Sf9 insect cells to overcome the interference between DA receptor subtypes. In this study, the effects of *l*-CSL on D_1 receptor were evaluated at the molecular and cellular level directly. For comparison, the D_1-selective agonist SKF38393 was included in the experiments.

MATERIALS AND METHODS

Drugs and reagents [^{3}H]SCH23390 (3.1 GBq/mol) and [^{3}H]spiperone (2.8 GBq/mol) were purchased from Amersham Pharmacia Co (USA). D_1 agonist SKF38393, non-selective antagonist (+)-butaclamol and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma (USA). *l*-12-Chloroscoulerine (*l*-CSL) was synthesized in Shanghai Institute of Materia Medica (China). cAMP radioimmunoassay kit was purchased from Shanghai Second Medical University (China).

Generation of recombinant baculovirus and expression of D_1 receptor The plasmid containing D_1R cDNA was a generous gift from Prof Kim NEVE (Oregon Health Sciences University, USA). The cDNA was digested from the plasmid with *Eco*R I and *Not* I, and then subcloned into the comparable sites of the transfer vector pVL1393. The recombinant baculovirus was generated by co-infection of Sf9 insect cells with pVL1393-D_1R and BaculoGold™ linearized baculovirus genomic DNA (Pharmingen, USA) and plaque purification. Sf9 insect cells were routinely cultured in monolayer at 27 °C in TNM-FH medium (Gibco BRL, USA) supplemented with 10 % heat-inactived fetal bovine serum (Gibco BRL, USA).

Receptor binding assay In saturation binding experiments, cells containing 20 μg protein were incubated with increasing concentration of [^{3}H]SCH23390 (0.1-9.0 nmol/L) in a final volume 200 μL binding buffer containing (in mmol/L) Tris-HCl 50, NaCl 120, KCl 5, $MgCl_2$ 5, $CaCl_2$ 1.5, edetic acid 5, pH7.4, at 30 °C for 40 min. For the competition binding experiments, the cells were incubated with [^{3}H]SCH23390 0.8 nmol/L in the presence of increasing amount of competing drugs. The nonspecific binding was determined by the addition of (+)-butaclamol 6 μmol/L.

CAMP assay Cells expressing D_1R were challenged with indicated agonists in the presence of 3-isobutyl-1-methylxanthine (IBMX) 500 μmol/L at 27 °C for 15 min. The reactions were terminated with perchloric acid 1 mol/L and neutralized with KOH 1 mol/L. The cAMP level of each sample was determined with radioimmunoassay kit as the protocol.

Data analysis Data were analyzed using Origin and Prism programs, and expressed as mean±SD obtained from 3 independent experiments.

RESULTS

Expression of D_1 receptor in the Sf9 insect cells After recombinant baculovirus AcNPV-D_1R infection, the morphologic change of Sf9 insect cells that cell body became bulgy, was observed as an index for expression of recombinant receptor (Fig 1). Expression of D_1 receptor was further monitored using the radioactive antagonist [^{3}H]SCH23390. Receptor expression approximately peaked at 72 h (Fig 2). [^{3}H]SCH23390 binding to D_1 receptor expressed in Sf9 cells was saturable and of high affinity, with Scatchard analysis indicating the presence of a homogenous binding site. The maximum binding amount (B_{max}) was (0.94±0.06) nmol/g protein. The equilibrium dissociation constant (K_d) of [^{3}H]SCH23390 was (1.9±0.3) nmol/L (Fig 3). The Sf9 cells expressing the D_1 receptor did not show the specific [^{3}H]spiperone binding to D_2-like receptors.

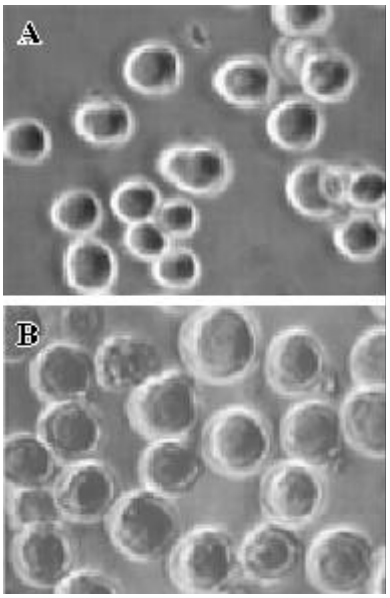


Fig 1. Morphologic change of Sf9 cells. ×400. A) normal cells; B) infected cells.

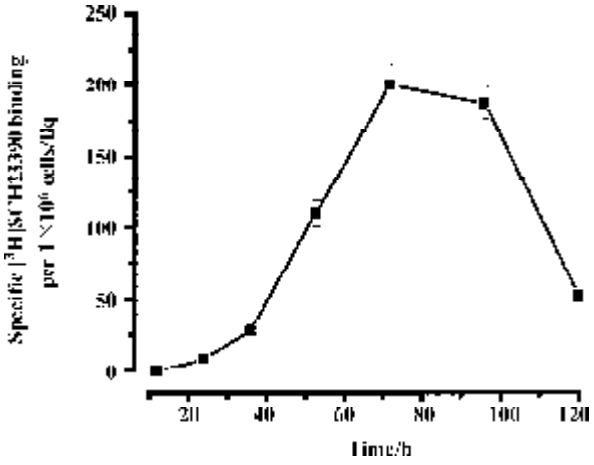


Fig 2. Time course of expression of D_1R in Sf9 cells. *n*=3. Mean±SD.

Affinity of *l*-CSL to recombinant D_1 receptor In receptor binding assay, *l*-CSL inhibited the [^{3}H] SCH23390 binding competitively to recombinant D_1 receptor with K_i (6.3±1.4) nmol/L, while D_1 agonist SKF38393 displaced the [^{3}H]SCH23390 binding with K_i (0.53±0.04) μmol/L (Fig 4).

Effect of *l*-CSL on cAMP level After a 15-min incubation with Sf9 cells expressing D_1 receptor, *l*-CSL challenged the increase of intracellular cAMP level in a dose-dependent manner with EC_{50} 0.72 μmol/L and 95% confidence limit was 0.67-0.77 μmol/L. SKF38893 increased intracellular cAMP level with EC_{50} 0.84 μmol/L and 95 % confidence limit was 0.51-1.40 μmol/L (Fig 5).

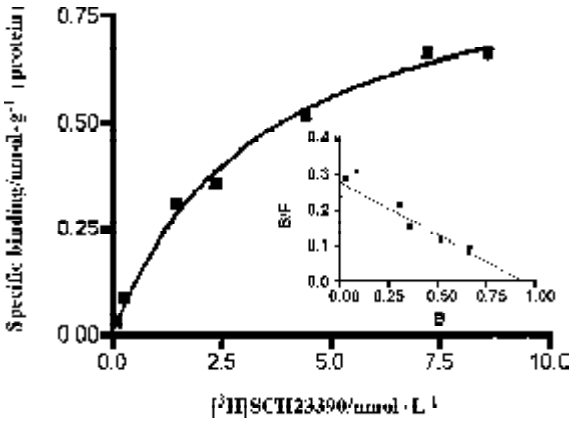


Fig 3. Saturation curve for the specific binding of [^{3}H]SCH23390 to Sf9 cells expressing D_1R. Inset, Scatchard analysis.

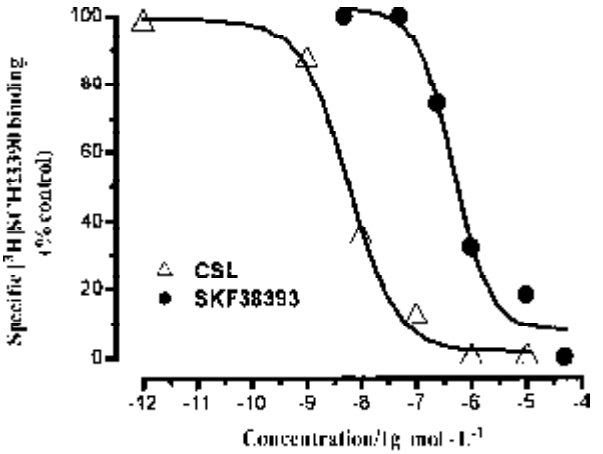


Fig 4. Competitive inhibition of binding of [^{3}H]SCH23390 to D_1R expressed in Sf9 cells by indicated drugs. The radioligand was used at a concentration of 0.8 nmol/L and non-specific binding was defined using (+)-butaclamol 6 mmol/L.

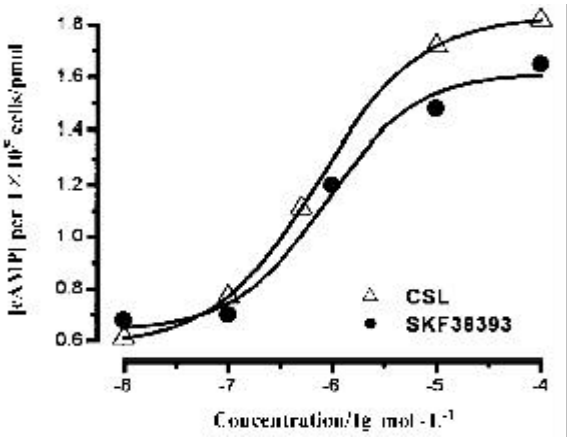


Fig 5. cAMP accumulation induced by *l*-CSL and SKF38393 in Sf9 cells expressing D_1R.

DISCUSSION

l-CSL showed high affinities for both D_1 and D_2 receptors in the binding assay with the calf striatum, and possessed D_1 agonistic-D_2 antagonistic dualactions[4]. In order to clarify its D_1 agonism, it is necessary to look for some tissue or cell line bearing D_1R only. Neither specific [^{3}H]SCH23390 binding to D_1-like receptors, nor specific [^{3}H]spiperone binding to D_2-like receptors was found in uninfected Sf9 insect cells in our preliminary experimental detection. Moreover, there were no endogenous 5-HT receptor[8], α-adrenergic receptor[9], and β-adrenergic receptor[10] in Sf9 insect cells. All of these indicated that the heterogenous baculovirus expression system met the need, and it could exclude the potential interference between the dopamine receptor subtypes or from other catecholamine receptors. Therefore, the baculovirus expression system was adopted here, which seemed an ideal model to assess the interaction between drug and dopamine receptor subtypes.

O' Dowd BF and his colleagues have expressed the dopamine D_1 receptor in Sf9 cell[11]. In order to detect the effect of *l*-CSL, we independently expressed the D_1R. The results of our recombinant D_1R binding assay were consistent with those of membrane preparation from calf striatum[1]. Compared with the results from calf striatum, the affinity of [^{3}H]SCH23390 to the recombinant D_1 receptor had no significant changes. The K_d value was (1.9±0.3) nmol/L in Sf9 cells, while the K_d value was 1.65 nmol/L in calf striatum. So, our recombinant D_1R maintained a native property, at least in our experiment.

D_1 receptor couples with G protein and adenylyl cyclase. D_1 receptor activation induces the cAMP level increase. As no other catecholamine receptors were found in Sf9 cells[8-10], the intracellular cAMP increase challenged by *l*-CSL was just induced by the recombinant D_1 receptor expressed in Sf9 cells.

This study showed the agonism of *l*-CSL on recombinant D_1 receptor directly, which was consistent with the previous observation of D_1 agonism of *l*-CSL in the 6-OHDA unilateral lesioned rats[4]. The data, taken with the previous results[1-4], confirmed that *l*-CSL possessed the D_1 agonistic-D_2 antagonistic dual actions. Both *l*-CSL and *l*-stepholidine (*l*-SPD) share the common active structural elements, dihydroxy groups, in the tetrahydroprotoberberines (so called DH-THPB), which result in the unique D_1 agonistic-D_2 antagonistic dual actions. The animal studies and clinical observations have indicated that dual effects of *l*-SPD may represent a novel antipsychotic drug[12]. In our study, *l*-CSL showed a more preferential affinity to recombinant D_1 receptor than *l*-SPD. The more potent dual actions were found compared to SPD in previous studies[4]. All data implicated that *l*-CSL would be a potential antipsychotic drug.

ACKNOWLEDGEMENT Authors thank Prof Kim NEVE (Oregon Health Sciences University, USA) for the generous gift of the plasmid containing D_1 receptor cDNA.

REFERENCES

1 Chen LJ, Xi Y, Pang DW, Zhou QT, Jin GZ. Effect of (±)12-chloroscoulerine on brain dopamine receptors. Acta Pharmacol Sin 1996; 17: 185-9.
2 Zhang XX, Jin GZ. (-)-Stepholidine *vs* 12-chloroscoulerine enantiomers on firing activity of substantia nigral dopamine neurons. Acta Pharmacol Sin 1996; 17: 18-22.
3 CHEN LJ, Zhang XX, Guo X. Blockade of (±)12-chloroscoulerine on feed-back regulation of dopamine D_2 autoreceptors. Acta Pharmacol Sin 1996; 17: 477-80.
4 Chen LJ, Zhou QT, Dong ZJ, Yu LP, Jin GZ. Comparison of 12-chloroscoulerine enantiomers on animal behavior to dopamine receptors. Acta Pharmacol Sin 1999; 20: 884-8.
5 White FJ, Bednarz LM, Wachtel SR, Hjorth S, Brooderson RJ. Is stimulation of D1 and D2 receptors necessary for the expression of dopamine-mediated behavors? Pharmacol Biochem Behav 1988; 30: 189-93.
6 Buonamici M, Caccia C, Carpentieri M, Pegrassi L, Rossi AC, DiChiara G. D-1 receptor supersensitivity in rat striatum after unilateral 6-hydroxydopamine lesions. Eur J Pharmacol 1986; 126: 347-8.
7 Porceddu ML, Giorgi O, DeMontis G, Mele S, Cocco L, Ongini E, *et al.* 6-Hydroxydopamine-induced degeneration of nigral dopamine neurons: differential effect on nigral and striatal D-1 dopamine receptors. Life Sci 1987; 41: 697-706.
8 Clawges HM, Depree KM, Parker EM, Graber SG. Human $5\text{-}HT_1$ recpetor sybtypes exhibit distinct G protein coupling behaviors in membranes from Sf9 cells. Biochemistry 1997; 36:12930-8.
9 Jansson CC, Karp M, Oker-Blom C, Nasman J, Savola JM, Akerman KE. Two human subtypes αA-C10 and αB-C2 expressed in Sf9 cells couple to transduction pathway resulting in opposite effects on cAMP production. Eur J Pharmacol 1995; 290: 75-83.
10 George ST, Arbabian MA, Ruoho AE, Kiely J, Malbon CC. High-efficiency expression of mammalian β-adrenergic receptors in baculovirus-infected insect cells. Biochem Biophys Res Commun 1989; 163: 1265-9.
11 Ng GY, Mouillac B, George SR, Caron M, Dennis M, Bouvier M, *et al.* Desensitization, phosphorylation and palmi-

toylation of the human dopamine D1 receptor. Eur J Pharmacol 1994; 267:7-19.

12 Jin GZ, Zhu ZT, Fu Y. *l*-Stepholidine: a potential novel antipsychotic drug with D_1 agonistic-D_2 antagonistic dual actions. Trends Pharmacol Sci 2002; 23: 4-7.