

도파민 발현 세포주 CV(bDAT) 세포에서 항정신병 약물이 도파민 수송체 활성화에 미치는 영향*

김영미*[†] · 이용성* · 이상훈* · 오동렬** · 양병환*** · 조석신**** · 남정현***

Effect of Antipsychotic Drugs on Dopamine Transporter Function in CV(bDAT) Cells*

Young-Mi Kim, Ph.D.,* Yong-Sung Lee, M.D., Ph.D.,* Sang-Hun Lee, M.D., Ph.D.,*
 Dong-Yul Oh, M.D., Ph.D.,** Byung-Hwan Yang, M.D., Ph.D.,***
 Suck-Shin Cho, M.D., Ph.D.,**** Jung-Hyun Nam, M.D., Ph.D.***

ABSTRACT

CV(bDAT) cell line, expressing dopamine transporter stably, has been established by transfection of CV-1 cells with bovine dopamine transporter cDNA. Using CV(bDAT) cells, the effects of various antipsychotic drugs on dopamine uptake activity were investigated. All of antipsychotic drugs tested, inhibited the [³H]dopamine uptake into CV(bDAT) cells with IC₅₀s in the low to mid micromolar range, implying that antipsychotic drugs may produce overflow of dopamine in the synaptic cleft of dopaminergic neuron.

KEY WORDS : Dopamine transporter · Dopamine uptake · Antipsychotic drug.

서론

synaptic terminal, synaptic cleft (dopamine transporter), post-synaptic neuron

receptor), pre-synaptic neuron, 60,000 (transmembrane domain), 5 (intracellular loop), 6 (extracellular loop), 2, N-terminus, C-terminus (Amara & Kuhar, 1993; Giros & Caron, 1993; Uhl & Hartig, 1992). Gilles de la Tourette syndrome, (tricyclic antidepressant) (Bergman, 1989; Ritz, 1987;

* 1993
 This study was supported by grant from the Clinical Research Laboratory of Hanyang University
 Department of Biochemistry, College of Medicine, Hanyang University, Seoul, Korea
 ** National Seoul Mental Hospital
 Department of Neuropsychiatry, College of Medicine & Mental Health Research Institute, Hanyang University, Seoul, Korea
 **** Department of Nuclear Medicine, College of Medicine, Hanyang University, Seoul, Korea
[†] : , 133 - 792, 17) (02) 290 - 8430,) (02) 294 - 2111

Spealman 1989).
 , self - admi -
 nistration (Bergman 1989 ; Ritz 1987)
 mesolimbic system
 (Kubar, 1992).
 monoamine
 가
 (Glowniak
 1993 ; Ramamoorthy 1993)
 GABA cDNA가 cloning
 (Guastella 1990) noradrenaline, serotonin
 cDNA가 cloning (Pacholczyk 1991 ; Bl -
 akely 1991 ; Hoffman 1991 ; Kilty 1991 ; Shimada
 1991 ; Usdin 1991)

glycol (Sambrook 1989) plasmid
 .
 60mm CV - 1 , 1 - 2 x 10⁵
 DMEM 18 24 가
 30 50% . OPTI - MEM I 100ul
 pRc/CMV - bDAT plasmid DNA가 2ug
 A OPTI - MEM I 100ul 10ul Lipofectin^R(BRL)
 B 15 .
 2ml OPTI - MEM I Li -
 pofectin - DNA 1.8ml OPTI - MEM I 가
 가 18 CO₂ . 4ml DM -
 EM 48 5
 Geneticin^R(BRL) 400ug/ml DMEM
 . Geneticin^R (colo -
 ny) 11 ,
 가 가 (CV(bDAT))

(antipsychotic drugs) postsynaptic neuron
 , serotonin, norepinephrine
 (receptor)
 가
 가
 cloning
 cDNA(Usdin 1991) CV - 1

2. 도파민 수송체 활성 측정
 , 24 - well plate
 가 transfection , 1 - 2 x 10⁵
 Geneticin^R DMEM
 modified Krebs - Ringer HEPES(m - KRH)
 [25mM HEPES(pH7.4), 125mM NaCl, 4.8mM KCl, 1.2
 mM KH₂PO₄, 1.2mM MgSO₄, 5.6mM glucose, 1mM CaCl₂,
 1mM ascorbic acid, 100uM pargyline] 0.5 ml
 10 37
 0.1, 0.2 0.5uM [³H]dopamine
 (51mCi/mMole, Amersham) , 100ul plate 가
 37 10 . Plate
 가 , 0.5ml 3
 [³H]dopamine . 0.5M NaOH
 , 100ul
 [³H]dopamine scintillation counter(Tri - Carb
 2010, Packard)
 가 CV - 1
 dopamine

연구대상 및 연구방법

1. 도파민 수송체 영구 발현 세포주 CV(bDAT)세포 제조
 cDNA(Usdin 1991) cytomegal -
 ovirus(CMV) promoter 가 eukaryotic expression vec -
 tor pRc/CMV(Invitrogen) multiple cloning site Hin -
 dIII NotI site expression plasmid pRc/C
 MV - bDAT , DH5 calcium
 chloride (Hanahan 1983) (transforma -
 tion) plasmid alkali lysis
 (Birnboim Doly 1979) polyethylene -

3. 항정신병 약물이 도파민 수송체 활성에 미치는 영향
 가 0.01uM 500uM 0.1uM
 [³H]Dopamine 가
 가 .
 가 50%

IC₅₀ 가 (CFT, GBR 12909, nomifensine, clomipramine, desipramine, imipramine, amineptine, amitriptyline, kinupril) 가 CFT, GBR12909, nomifensine, clomipramine, desipramine, imipramine, amineptine, amitriptyline, kinupril, clozapine, risperidone, haloperidol, bromperidol, clopixol, mesoridazine, thioridazine, chlorpromazine Research Biochemical International(RBI)

결 과

1. 소 도파민 수송체의 활성 및 특성

CV(b DAT) , 50nM [³H]dopamine 가 37 10 1.28pmol/10⁵ cell [sup3H]dopamine , saturation analysis K_m (Michaelis constant) 991.6nM (Fig. 1).

2. 항 정신성 약물이 도파민 수송능력에 미치는 영향

CFT IC₅₀ 가 0.27uM 가

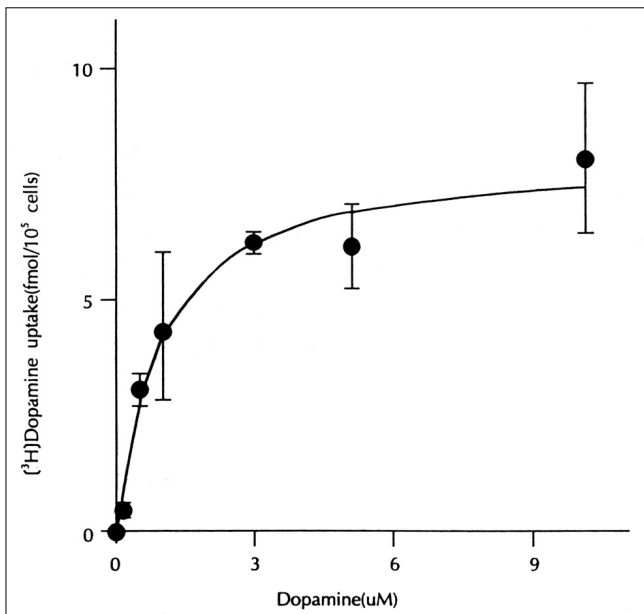


Fig. 1. Saturation analysis of [³H]dopamine uptake in CV(bDAT) cells. Cells were preincubated in a modified-KRH buffer at 37 for 10 min. Dopamine uptake was assessed by incubation with 50 nM [³H]dopamine and unlabeled dopamine (0.5 - 100uM) at 37 for 10 min. Data are the mean ± standard error from three experiments.

가 GBR12909 0.45uM . nomifensine IC₅₀ 가 0.86uM 가 , amitriptyline 4.01uM, clomipramine 4.64uM, imipramine 7.35uM, desipramine 9.82uM , kinupril amineptine 21.86uM, 52.22uM (Table 1).

3. 항 정신병 약물이 도파민 수송능력에 미치는 영향

가 thioridazine IC₅₀ 가 3.44uM br - omperidol 6.59uM imipramine de - sipramine 가 , clozapine mesoridazine IC₅₀ 96.34uM, 165.97uM 가 가 . cl - opixol 9.85uM, haloperidol 15.83uM, risperidone 17.38uM, chlorpromazine 17.52uM (Table 1, Fig. 2).

Table 1. Effects of psychotropic and antipsychotic drugs on [³H]Dopamine uptake in CV(bDAT) cells expressing bovine dopamine transporter

Classification	Action site	Drug	IC ₅₀ * (uM)	
Cocaine analogs	Dopamine transporter	CFT	0.27	
		GBR12909	0.45	
Antidepressant	Monoamine transporter	Nomifensine	0.86	
		Clomipramine	4.64	
		Imipramine	7.35	
		Desipramine	9.82	
		Amineptine	52.22	
		Amitriptyline	4.01	
Neuroleptics	serotonine receptor	Kinupril	21.86	
		Clozapine	96.34	
	dopamine receptor	Risperidol	17.38	
		Haloperidol	11.83	
	dopamine and norepinephrine receptor	Bromperidol	6.59	
		Clopixol	9.85	
	norepinephrine receptor	Mesoridazine	165.97	
		Thioridazine	3.44	
			Chlorpromazine	17.52

Assay was carried out under the presence of 0.1uM [³H]dopamine with 0.01 - 500 uM of each test drug at 37

*Concentration of drug at which 50% of [³H]dopamine uptake was inhibited

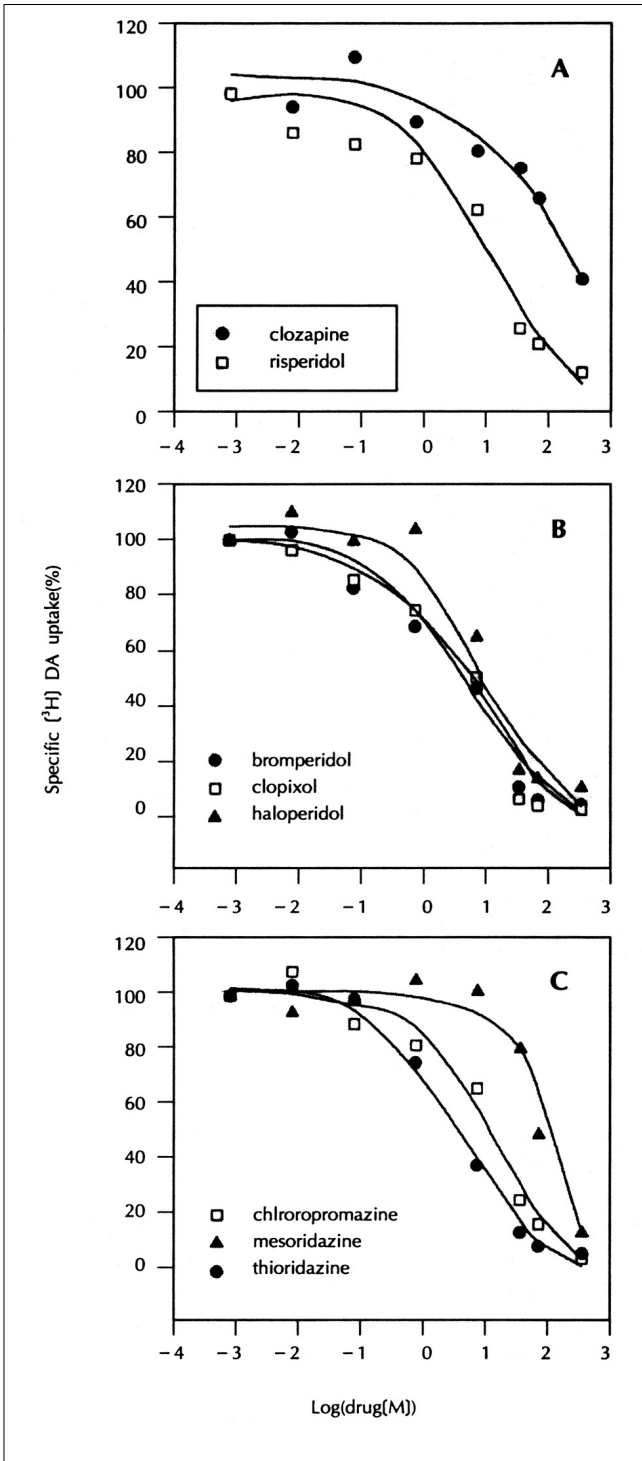


Fig. 2. Inhibition of [³H]dopamine uptake cells by antipsychotic drugs. A, serotonergic antipsychotic drugs whose major site of action are serotonin receptor ; B, dopaminergic antipsychotic drugs ; C, norepinephrinergic antipsychotic drugs. DAT-expressing CV (bDAT) cells were incubate with 0.1uM [³H]dopamine in the presence of 0.01 - 500uM of test drug at 37 for 10 min, then uptake values were determined as described in ' Materials and Methods '. Values in representative curves reflect the means of three determinations.

고찰

[³H]dopamine 가
 [³H]dopamine 가
 125mM Na
 mono-
 amine monoamine oxidase
 pargyline 가 [³H]dopamine
 (CFT)
 K_m
 991.6nM
 cDNA HeLa cell transfection Kilty (Kilty
 1991) K_m 885nM
 (Horn AS, 1990 ; Kilty 1991 ; Shimada
 1991 ; Usdin 1991 ; Giros 1992).
 GBR12909 CFT, no -
 mifensine, clomipramine, imipramine, desipramine, amine -
 ptine, amitrypyline kinupril
 가
 가
 CFT, GBR12909, nomifensine 가
 , amitrypyline, clomipramine, imipramine,
 desipramine, kinupril, amineptine 가 가
 Usdin (Usdin 1991)
 가
 norepinephrine
 thioridazine IC₅₀ 가 3.44uM
 amitrypyline 4.01uM
 bromperidol
 mesoridazine clozapine IC₅₀ 가
 165.87uM, 96.34uM
 가

kinetics ,
 V_{max} K_m 가
 가
 Na^+
 Na^+
 Na^+
 (Shimada 1991).
 Na^+ 가 ouabain
 Na^+ , K^+ - ATPase
 (Holz Coyle 1974 ; Sirinathsinghji 1988).
 Kitayama(Kitayama 1994) Lee(Lee 1996)
 C(protein kinase C)
 가 가 amphetamine
 (Pifl
 1995 ; Wall 1995 ; Eshleman 1993),
 가
 가
 synaptic cleft (overflow)
 (tardive dyskinesia)
 결 론
 CV(bDAT)

1) CV
 (bDAT) 가
 , saturation analysis
 K_m 991.6nM
 2) 가
 가 ,
 CFT GBR12909
 nomifensine 가
 , amitriptyline, clomipramine, imipramine,
 desipramine, kinupril, amineptine
 3)
 , thioridazine, bromperidol
 가 , clop-
 ixol, haloperidol, risperidone, chlorpromazine, clozapine, me-
 soridazine

참고문헌

Amara SG, Kuhar MJ(1993) : Neurotransmitter transporters. *Recent progress. Annu Rev Neurosci* 16 : 73-93

Bergman J, Madras BK, Johnson SE, Spealman RD(1989) : Effects of cocaine and related drugs in nonhuman primates III. self-administration by squirrel monkeys. *J Pharmacol Exp Ther* 251 : 150-155

Birnboim HC, Doly J(1979) : A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res* 7 : 1513

Blakely RD, Berson HE, Freneau RT, Caron MG, Peek MM(1991) : Cloning and expression of a functional serotonin transporter from rat brain. *Nature* 354 : 66-70

Eshleman AJ, Henningsen RA, Neve KA, Janowsky A(1993) : Release of dopamine via the human transporter. *Mol pharmacol* 45 : 312-316

Giros B, Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, Caron MG(1992) : Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol* 42 (3) : 383-390

Giros B, Caron MG(1993) : Molecular characterization of dopamine transporter. *Trend in Pharm Sci* 14 : 43-49

Glowniak JV, Kilty JE, Amara SG, Hoffman BJ, Turner FE(1993) : Evaluation of metaiodobenzylguanidine uptake by the norepinephrine, dopamine, and serotonin transporter. *J Nuc Med* 34 : 1140-1146

Guastella J, Nelson N, Nelson H, Czyzk L, Keynan S(1990) : Clo-

- ning and expression of a rat brain GABA transporter. *Science* 249 : 1303-1306
- Hanahan D(1983)** : Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166 : 557
- Hoffman BJ, Mezby E, Brownstein MJ(1991)** : Cloning of a serotonin transporter affected by antidepressants. *Science* 254 : 79-80
- Holz RW, Coyle JT(1988)** : The effect of various salts, temperature and the alkaloids veratridine and batrachotoxine on the uptake of [³H]dopamine into synaptosomes from rat striatum. *Mol Pharmacol* 10 : 746-758
- Horn AS(1990)** : Dopamine uptake: a review of progress in the last decade. *Prog Neurobiol* 34 : 387-400
- Kilty JE, Lorang D, Amara SG(1991)** : Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science* 254 : 578-579
- Kitayama S, Dohi T, Uhl GR(1994)** : Phorbol esters alter function of the expressed dopamine transporter. *Eur J Pharmacol* 268 : 115-119
- Kubar MJ(1992)** : Molecular pharmacology of cocaine a dopamine hypothesis and its implication. *Ciba Found Symp* 166 : 81-89
- Lee SH, Koh JK, Han JS, Kim YM, Lee JH, Lee YS(1996)** : Modulation of dopamine transporter function by protein kinase C. *Exp Mol Medicine* 28 : 85-88
- Pacholczyk T, Blakely RD, Amara SG(1991)** : Expression cloning of a cocaine-and antidepressant-sensitive human noradrenaline transporter. *Nature* 350 : 350-353
- Piffl C, Drobny H, Reither H, Hornykiewicz O, Singer EA(1995)** : Mechanism of the dopamine-releasing actions of amphetamine and cocaine : plasmalemmal dopamine transporter versus vesicular monoamine transporter. *Mol Pharmacol* 47 : 368-373
- Ramamoorthy S, Leibach FH, Mahesh VB, Ganapathy V(1993)** : Partial purification and characterization of the human placental serotonin transporter. *Placenta* 14 : 449-461
- Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ(1987)** : Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237 : 1219-1223
- Sambrook J, Fritsch EF, Maniatis T(1989)** : In *Molecular cloning. a laboratory manual*. Cold Spring Harbor Laboratory Press 2ed : 1.40-1.41
- Shimada S, Kitayama S, Lin CL, Patel A, Nanthakumar E, Gregor P, Kuhar M, Uhl G(1991)** : Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* 254 : 576-578
- Sirinathsinghji DJS, Heavens RP, Sikdar SK(1988)** : In vivo studies on the dopamine re-uptake mechanism in the striatum of the rat : effects of benztropine, sodium and ouabain. *Brain Res* 438 : 399-403
- Spealman RD, Madras BK, Bergman J(1989)** : Effects of cocaine and related drugs in nonhuman primates II. Stimulant effects on schedule-controlled behavior. *J pharmacol Exp Ther* 261 : 142-149
- Uhl GR, Hartig PR(1992)** : Transporter explosion : update on uptake. *Trends Pharmacol Sci* 13 : 421-425
- Usdin TB, Mezey E, Chen C, Brownstein MJ, Hoffman BJ(1991)** : Cloning of the cocaine-sensitive bovine dopamine transporter. *Proc Natl Acad Sci USA* 88 : 11168-11171
- Wall SC, Gu H, Rudnick G(1995)** : Biogenic amine flux mediated by cloned transporters stably expressed in cultured cell lines : amphetamine specificity for inhibition and efflux. *Mol Pharmacol* 47 : 544-550