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Structure–Activity Relationship and Evaluation of Phenethylamine and Tryptamine Derivatives for Affinity towards 5-Hydroxytryptamine Type 2A Receptor

Shujie Wang[1], Anlin Zhu[1], Suresh Paudel[1], Choon-Gon Jang[2], Yong Sup Lee[3] and Kyeong-Man Kim[1,*]

[1]Pharmacology Laboratory, College of Pharmacy, Chonnam National University, Gwangju 61146,
[2]Pharmacology Laboratory, College of Pharmacy, Sungkyunkwan University, Suwon 16419,
[3]Medicinal Chemistry Laboratory, Department of Pharmacy, College of Pharmacy, Kyung Hee University, Seoul 02447, Republic of Korea

Abstract

Among 14 subtypes of serotonin receptors (5-HTRs), 5-HT_{2A}R plays important roles in drug addiction and various psychiatric disorders. Agonists for 5-HT_{2A}R have been classified into three structural groups: phenethylamines, tryptamines, and ergolines. In this study, the structure-activity relationship (SAR) of phenethylamine and tryptamine derivatives for binding 5-HT_{2A}R was determined. In addition, functional and regulatory evaluation of selected compounds was conducted for extracellular signal-regulated kinases (ERKs) and receptor endocytosis. SAR studies showed that phenethylamines possessed higher affinity to 5-HT_{2A}R than tryptamines. In phenethylamines, two phenyl groups were attached to the carbon and nitrogen (R^3) atoms of ethylamine, the backbone of phenethylamines. Alkyl or halogen groups on the phenyl ring attached to the β carbon exerted positive effects on the binding affinity when they were at para positions. Oxygen-containing groups attached to R^3 exerted mixed influences depending on the position of their attachment. In tryptamine derivatives, tryptamine group was attached to the β carbon of ethylamine, and ally groups were attached to the nitrogen atom. Oxygen-containing substituents on large ring and alkyl substituents on the small ring of tryptamine groups exerted positive and negative influence on the affinity for 5-HT_{2A}R, respectively. Ally groups attached to the nitrogen atom of ethylamine exerted negative influences. Functional and regulatory activities of the tested compounds correlated with their affinity for 5-HT_{2A}R, suggesting their agonistic nature. In conclusion, this study provides information for designing novel ligands for 5-HT_{2A}R, which can be used to control psychiatric disorders and drug abuse.

Key Words: 5-HT_{2A} receptor, Phenethylamine, Tryptamine, Structure activity relationship, ERK, Endocytosis

INTRODUCTION

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) controls numerous physiological functions of the central and peripheral nervous systems, including sleep/wake cycle, food intake, nociception, locomotion, and cardiovascular homeostasis (Darmon *et al*., 2015).

5-HT receptors (5-HTRs) are classified into seven distinct subfamilies, 5-HT_{1-7}R. All 5-HTR subtypes belong to G protein-coupled receptor (GPCR) superfamily, except for 5-HT_3R, which is a ligand-gated ion channel (Yun and Rhim, 2011).

The type 2 serotonin receptor subfamily contains 5-HT_{2A}R, 5-HT_{2B}R, and 5-HT_{2C}R (Bonhaus *et al*., 1995; Leysen, 2004). Among these subtypes, 5-HT_{2A}R is expressed widely throughout the central nervous system that includes neocortex (mainly prefrontal, parietal, and somatosensory cortex) and the olfactory tubercle. High concentrations of this receptor on the apical dendrites of pyramidal cells in layer V of the cortex play a key role in several diseases such as drug addiction (Krebs and Johansen, 2012), schizophrenia (Vollenweider *et al*., 1998), obsessive compulsive disorder (Adams *et al*., 2005; Moreno *et al*., 2006), depression (Celada *et al*., 2004; Carhart-Harris *et al*., 2012), and neuropathic pain (Okamoto *et al*., 2007).

5-HT_{2A}R agonists have traditionally been divided into three structural groups: phenethylamines, tryptamines, and ergolines (Nichols, 2012). Phenethylamines have been most extensively characterized (Parker *et al*., 1998; McLean *et al*., 2006). They generally show selectivity for 5-HT_{2A}R; however,

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***Corresponding Author**
E-mail: kmkim@jnu.ac.kr
Tel: +82-62-530-2936, Fax: +82-62-530-2949

they also bind to 5-HT_{2C}R with high affinity (Nelson *et al*., 1999).

Tryptamines with tryptophan ring as the basal structural moiety are structurally close to 5-HT, the endogenous transmitter. Tryptamines are more selective and have stronger affinity than ergolines for 5-HT_{2A}Rs. Tryptamine derivatives include ring substituents, *N*-alkylation, and side chain alkylation (Nichols, 2012).

Ergolines are tetracyclic molecules derived from alkaloids produced by the ergot fungus. Ergolines are considered to be rigidified tryptamines; however, they generally show little subtype selectivity compared to that of phenethylamines and tryptamines (Nelson *et al*., 1999). Ergolines are structurally complex, and deriving their structural analogs is difficult.

In this study, we conducted radioligand binding study on 5-HT_{2A}R with 11 tryptamines and 14 phenethylamine derivatives. The affinity for 5-HT_{2A}R was largely determined by scaffolds, and the details were determined by the size or inclusion of the side branches. The relative efficacies and potencies of 5-HT agonists were in accordance with their affinity for 5-HT_{2A}R as evident by the activation of ERK signaling and receptor endocytosis. The information obtained here will be useful in developing 5-HT_{2A}R ligands optimized for therapeutic purposes and avoiding side effects, such as psychedelic actions.

MATERIALS AND METHODS

Reagents

5-HT, ketanserin (+)-tartrate, 2-bromo-α-ergocryptine, and rabbit anti-hemagglutinin (HA) were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Peroxidase-conjugated anti-rabbit antibodies were purchased from Invitrogen (Waltham, MA, USA). TMB (3,3',5,5'-Tetramethylbenzidine)-ELISA substrate was procured from Thermo Fisher Scientific (Waltham, MA, USA). Methylspiperone (84.2 Ci/mmol) was purchased from PerkinElmer Life Sciences (Waltham, MA, USA). Antibodies to phospho-ERK1/2 and ERK2 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti-mouse horse peroxidase (HRP)-conjugated secondary antibodies were obtained from Jackson ImmunoResearch (West Grove, PA, USA). Phenethylamine, tryptamine, and their derivatives were provided by Korean Ministry of Food and Drug Safety (Cheongju, Korea).

Plasmid constructs

5-HT_{2A}R in pCNS-D2 was provided from Korea Human Gene Bank, Medical Genomics Research center, KRIBB, Daejeon, Korea. 5-HT_{2A}R was tagged either with FLAG or HA at amino terminal. Wildtype and dominant negative mutants of dynamin2 (K44A-Dyn2) were described previously (Henley *et al*., 1998; Guo *et al*., 2015).

Cell culture

Human embryonic kidney (HEK-293) cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in a humid environment containing 5% CO2, using a minimal essential medium containing 8% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were transfected using polyethylenimine (Polysciencies Inc., Warrington, PA, USA).

5-HT_{2A}R binding assay

HEK-293 cells expressing 5-HT_{2A}R were sub-seeded in a 24-well plate coated with poly-L-lysine. Radioligand binding assay was performed by incubating cells with 1 nM [^{3}H]-methylspiperone at 4°C for 90 min. Cells were washed three times with ice-cold serum-free media and then lysed with phosphate-buffered saline (PBS) containing 1% sodium dodecyl sulfate (SDS). Radioactivity was determined by Wallac 1450 MicroBeta® TriLux liquid scintillation counter (PerkinElmer Life Sciences). The binding of [^{3}H]-methylspiperone in the presence of 10 μM ketanserin was defined as non-specific. Preparation of dose–response curves and statistical analysis were performed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). Ki values were converted from IC_{50} values according to the following Cheng-Prusoff equation (Cheng and Prusoff, 1973), $Ki=IC_{50}/(1+[A]/Kd)$ where [A] and Kd represent the concentration of the compound used and dissociation constant of [^{3}H]-methylspiperone for 5-HT_{2A}R, respectively.

ERK measurement

Transfected cells were cultured in 6-well plates, and were starved overnight in a serum-free culture medium containing 0.1% bovine serum albumin (BSA). Cells were treated with 5-HT dissolved in serum-free culture medium, and sodium dodecyl sulfate (SDS) sample buffer was directly added to culture wells. After incubating for 20 min at 65°C, samples were sonicated to shear genomic DNA. Proteins were separated by SDS-polyacrylamide gel electrophoresis (10% running gel, 5% stacking gel) and electroblotted onto polyvinylidene difluoride or nitrocellulose membranes. The membranes were incubated for 1 h at 22°C in TBS-Tween 20 (TBS-T) containing 5% nonfat dry milk or 4% BSA, followed by 1 h of incubation with antibody to phospho-ERK (1:1,000 dilution) and 1 h with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000 dilution) in 2% nonfat dry milk. Blots were visualized with chemiluminescent western blotting kit. The same samples were processed to detect ERK. Signals were quantified using Multi Gauge version 3.1 (FUJIFILM Corporation, Tokyo, Japan).

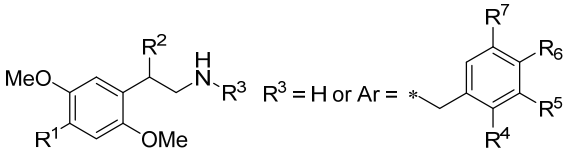
Receptor endocytosis assay

Endocytosis of 5-HT_{2A}R was determined by enzyme-linked immunosorbent assay (ELISA). HEK-293 cells were transfected with either HA-tagged 5-HT_{2A}R. Cells were treated with 1 μM 5-HT for a designated time period and washed with PBS three times. Cells were fixed with 4% paraformaldehyde for 15-20 min on ice and washed with PBS three times and then treated with 1% BSA for 1 h, followed by incubation with anti-HA or ant-FLAG antibodies (1:2,000 dilution in 1% BSA) for 1 h. Cells were then washed with PBS three times and treated with of HRP-conjugated anti-rabbit secondary antibody (1:2,000 dilution in 1% BSA) for 1 h at 20°C. After washing with PBS three times, cells were treated with TMB substrate solution for at least 10 min, and the reaction was stopped by adding sulfuric or phosphoric acid. Optical density was monitored at 450 nm.

Statistical analysis

Values are expressed as the mean ± standard deviation. Statistical significance of the data was analyzed using a one-way analysis of variance with Tukey's post-hoc test using

Table 1. Binding affinity of phenethylamine derivatives for 5-HT_{2A}R.



Compd	R^1	R^2	R^3	R^4	R^5=R^6=R^7	K_i (nM)
1	-$CH_2CH_2CH_3$	-H	-H	-H	-H	3.473
2	-NO_2	-H	-H	-H	-H	25.17
3	-$OCH_2C(CH_3)=CH_2$	-H	-H	-H	-H	71.92
4	-Cl	-H	-H	-H	-H	13.04
5	-CH_3	-OCH_3	-H	-H	-H	1.907
6	-CH_3	-H	-Ar	-OCH_3	-H	2.881
7	-CH_2CH_3	-H	-Ar	-OCH_3	-H	1.524
8	-Cl	-H	-Ar	-OCH_3	-H	0.817
9	-Br	-H	-Ar	-OCH_3	-H	1.411
10	-Cl	-H	-Ar	-OH	-H	1.099
11	-Cl	-H	-Ar	-F	-H	10.84
12	-Br	-H	-Ar	-F	-H	3.629
13	-I	-H	-Ar	-F	-H	10.00
14	-Cl	-H	-Ar	-H	-OCH_3	515.1
Ketanserin						22.21

1. 2C-P HCl: 2,5-dimethoxy-4-propyl-benzeneethanamine, HCl.
2. 2C-N HCl: 2,5-Dimethoxy-4-nitrophenethylamine, HCl.
3. Methallylescaline HCl.
4. 2C-C HCl: 4-Chloro-2,5-Dimethoxyphenethylamine, HCl.
5. BOD HCl: 4-methyl-2,5,beta-trimethoxyphenethylamine, HCl.
6. 25D-NBOMe HCl: 2-(2,5-dimethoxy-4-methylphenyl)-N-(2-methoxybenzyl)ethanamine, HCl.
7. 25E-NBOMe HCl: 2-(4-ethyl-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine, HCl.
8. 25C-NBOMe HCl: 2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine, HCl.
9. 25B-NBOMe HCl: 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine, HCl.
10. 25C-NBOH HCl: 2-[[[2-(4-chloro-2,5-dimethoxyphenyl)ethyl]amino]methyl]-phenol, HCl.
11. 25C-NBF HCl: 4-chloro-N-[(2-fluorophenyl)methyl]-2,5-dimethoxy-benzeneethanamine, HCl.
12. 25B-NBF HCl: 4-bromo-N-[(2-fluorophenyl)methyl]-2,5-dimethoxy-benzeneethanamine, HCl.
13. 25I-NBF HCl: N-(2-fluorobenzyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine, HCl.
14. 30C-NBOMe HCl: 2-(4-chloro-2,5-dimethoxyphenyl)-N-(3,4,5-trimethoxybenzyl)ethanamine, HCl.

GraphPad Prism 5.0. A *p*-value<0.05 was considered significant.

RESULTS

Characterization of phenethylamine derivatives in 5-HT_{2A}R binding

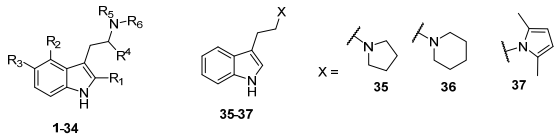
Phenethylamine is a primary amine in which the amino group is attached to a benzene ring through a two-carbon or ethyl group (Table 1, upper panel). Radioligand binding assay on 5-HT_{2A}R was conducted with 14 phenethylamine derivatives using ketanserin as a positive control (Glennon *et al*., 2002). The structure-activity relationship (SAR) was determined by classifying them according to the substitutions in seven positions (R^1–R^7, Table 1).

The affinity for 5-HT_{2A}R was maintained at similar range when R^1 attached to the para position of the phenyl ring contained an alkyl or a halogen group (**1,4, 6-9, 11-13**). In contrast, the affinity of the compounds decreased when alkoxy or nitro group was placed at R^1 (**2, 3**). A methoxy group at R^2 did not exert noticeable impact on the binding affinity to 5-HT_{2A}R (**1** *vs* **5**). An aromatic group at R^3 increased the binding affinity of phenethylamine to 5-HT_{2A}R if the aromatic ring at R^3 contained oxygen-containing group at the ortho position (R^4) without exception but at less extent with fluoride at R^4 (**6–10** *vs* **1–4** or **11–13**). The presence of methoxy groups at R^5–R^7 that correspond to meta and para positions, exerted negative effects on their affinity to 5-HT_{2A}R (**14**).

Characterization of tryptamine derivatives in 5-HT_{2A}R binding

Tryptamine is composed of an indole (benzene ring plus pyrrole ring) and a 2-aminoethyl group that is attached to the third carbon of pyrrole ring. For SAR analysis, IC_{50} values of 11 compounds, which were determined from our study, were

Table 2. Binding affinity of tryptamine derivatives for 5-HT_{2A}R.



Compd	Aromatic ring substitution			Side chain Alkylation	N-Alkylation		K_i (nM)
	R^1	R^2	R^3	R^4	R^5	R^6	
1	$-CH_3$	-H	-H	-H	-H	-H	685.70
2[A]	-H	-H	-H	$-CH_3$	-H	-H	130
3	-H	-H	$-OCH_3$	$-CH_3$	-H	-H	3.121
4	-H	-H	-Cl	$-CH_3$	-H	-H	23.97
5	-H	-H	-Br	$-CH_3$	-H	-H	58.78
6	-H	-H	-H	$-CH_2(CH_2)_2CH_3$	-H	-H	123.70
7[A]	-H	-H	-H	$-CH_3$	-H	$-CH_3$	46
8[C]	-H	-H	$-OCH_3$	-H	-H	$-CH(CH_3)_2$	50
9	-H	-H	-H	-H	$-CH_3$	$-CH_3$	88.49
10[A]	-H	-OH	-H	-H	$-CH_3$	$-CH_3$	6
11[B]	-H	-H	-OH	-H	$-CH_3$	$-CH_3$	3.5
12[A]	-H	-H	$-OCH_3$	-H	$-CH_3$	$-CH_3$	14
13	-H	-OH	-H	-H	$-CH_3$	$-CH_2CH_3$	64.8
14[C]	-H	-H	-H	-H	$-CH_3$	$-CH(CH_3)_2$	38
15[C]	-H	-H	$-OCH_3$	-H	$-CH_3$	$-CH(CH_3)_2$	17
16[C]	-H	-H	$-CH_3$	-H	$-CH_3$	$-CH(CH_3)_2$	28
17[C]	-H	-OH	-H	-H	$-CH_3$	$-CH(CH_3)_2$	13
18	-H	-OAc	-H	-H	$-CH_2CH_3$	$-CH_2CH_3$	595.30
19[C]	-H	-OH	-H	-H	$-CH_2CH_3$	$-CH_2CH_3$	14
20	-H	-H	$-OCH_3$	-H	$-CH_2CH_3$	$-CH_2CH_2CH_3$	122.30
21[B]	-H	-H	-OH	-H	$-CH(CH_3)_2$	$-CH(CH_3)_2$	5.6
22[D]	-H	-H	-H	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	701
23[D]	-H	-OAC	-H	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	565
24[D]	-H	-OH	-H	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	652
25	-H	-H	$-OCH_3$	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	258.41
26[D]	$-CH_3$	-H	$-OCH_3$	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	1,153
27[D]	-H	-H	F	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	247
28[D]	-H	-H	Br	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	477
29[D]	$-CH_3$	-H	F	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	655
30[D]	$-CH_2CH_3$	-H	-H	-H	$-CH_2CH=CH_2$	$-CH_2CH=CH_2$	1,515
31[A]	-H	-OH	-H	-H	$-CH_2CH(CH_3)_2$	$-CH_2CH(CH_3)_2$	260
32[A]	-H	-OH	-H	-H	$-CH_2CH(CH_3)CH_2CH_3$	$-CH_2CH(CH_3)CH_2CH_3$	39
33	-H	-H	-H	-H	$-CH_2(CH_2)_2CH_3$	$-CH_2(CH_2)_2CH_3$	822.90
34[A]	-H	-H	-H	-H	Pyrrolidinyl		110
35[A]	-H	-H	-H	-H	Piperidyl		760
36[C]	-H	-H	-H	-H	2,5-dimethylpyrol		2,430
Ketanserin							22.21

[A]These were adapted from (Nichols, 2012). [B]This was adapted from (Klein *et al*., 2011). [C]These were adapted from (McKenna *et al*., 1990). [D]These were adapted from (Klein *et al*., 2018).

1. 2-MT.

3. 5-MeO-AMT: 5-methoxy-α-methyltryptamine.

4. 5-CAMT HCl: 5-Chloro-3-(2-aminoethyl)indole Hydrochloride.

5. 5-BAMT HCl: 5-Bromo-3-(2-aminoethyl)indole Hydrochloride.

6. ABT HCl.

9. DMT fumarate: N,N-dimethyl-1H-indole-3-ethanamine, (2E)-2-butenedioate.

13. 4-OH-MET fumarate: 4-hydroxy-N-methyl-N-ethyltryptamine, metocin, or methylcybin.

18. 4-AcO-DET fumarate (1 : 0.83): 4-Acetoxy-N,N-diethyltryptamine.

20. 5-MeO-EPT: 5- methoxy -N-ethyl-N-propyl tryptamine.

25. 5-MeO-DALT: N-allyl-N-[2-(5-methoxy-1H-indol-3-yl)ethyl] prop-2-en-1- amine.

33. DBT HCl: 1H-Indole-3-ethanamine, N,N-dibutyl-N,N-Dibutyltryptamin.

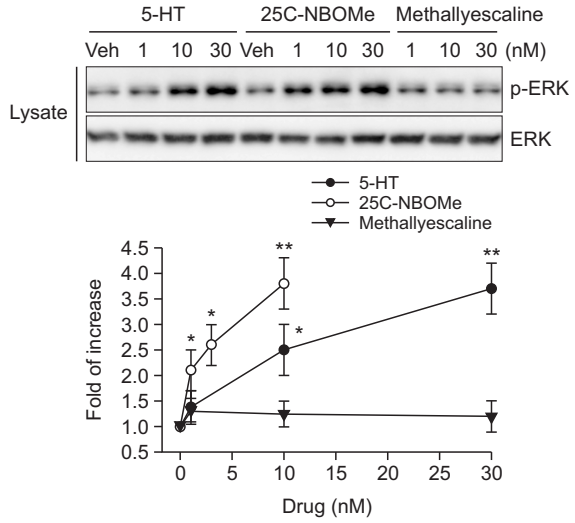


Fig. 1. Effects of 5-HT_{2A}R agonists on ERK activation. HEK-293 cells were transfected with 5-HT_{2A}R cDNA in pCNS-D2. Cells were treated with increasing concentrations of 5-HT, 25C-NBOMe, and methallyescaline for 5 min. 5-HT was dissolved in serum-free media; 25C-NBOMe and methallyescaline were dissolved in DMSO. **p*<0.05, ***p*<0.01 compared with the vehicle-treated group (n=3).

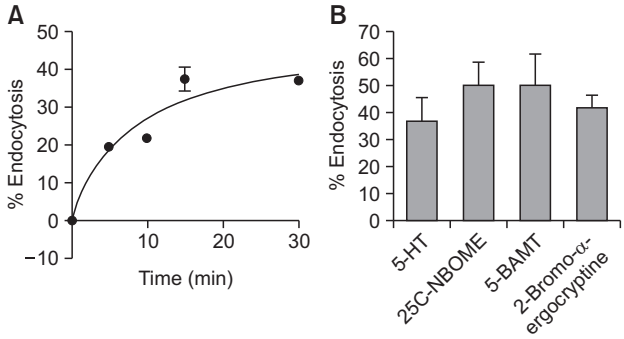


Fig. 2. Effects of 5-HT_{2A}R agonists on receptor endocytosis. (A) HEK-293 cells were transfected with HA-tagged 5-HT_{2A}R cDNA in pRC/CMV. Cells were treated with 1 μM 5-HT for 0-30 min. Receptor endocytosis was studied as described in Materials and Methods part. (B) HEK-293 cells were transfected with HA-tagged 5-HT_{2A}R cDNA in pRC/CMV. Cells were treated with 1 μM of 5-HT, 25C-NBOME, 5-BMAT, or 2-bromo-α-ergocryptine for 15 min.

combined with the IC_{50} values of 25 other compounds that were cited from previous studies (Table 2). SAR analysis was focused on three parts of tryptamine—indole ring, side chain alkylation, and N-alkylation. In addition, cyclic amino groups, such as pyrrolidinyl, piperidyl, and 2,5-dimethylpyrol, at N-alkylation site (**34–36**) were included.

The presence of alkyl group at R^1 exerted negative effects on the binding affinity (**1, 26, 29,** and **30**). The presence of hydroxyl group at R^2 exerted favorable effects on the binding affinity without exception (**9** *vs* **10, 14** *vs* **17, 22** *vs* **24,** and **31** *vs* **33**). The presence of any substitution at R^3 exerted positive influences on the binding affinity for 5-HT_{2A}R regardless of the nature of substituents being electron-donating (-CH_3, -OH, and -OMe; **2** *vs* **3, 9** *vs* **11** and **12,** and **14** *vs* **15** and **16**) or electron-withdrawing (-Cl and -Br; **2** *vs* **4** and **5,** and **22** *vs* **27** and **28**). In various formats of side chains, the presence of allyl groups at R^5 and R^6 exerted strong inhibitory effects on their affinity for 5-HT_{2A}R (**22–30**). In general, less bulky alkyl groups at R^5 and R^6 exerted more favorable influences on the affinity of compounds in the format of either no substitutions at R^1–R^3 (**9, 14, 22,** and **33**) or OH group at R^2 (**10, 13, 17, 19, 24,** and **31**).

Functional characterization of 5-HT_{2A}R agonists

The functionality of 5-HT_{2A}R ligands was tested by evaluating their effects on 5-HT_{2A}R-mediated ERK activation. Three compounds, including 5-HT (Ki, 11.55 nM) as a positive control, 25C-NBOMe (Ki, 0.817 nM), and methallyescaline (Ki, 71.92 nM) were selected for ERK assay. As shown in Fig. 1, the compounds induced ERK activation in a dose-dependent manner. Their effects on ERK activation were in agreements with their affinity towards 5-HT_{2A}R, that is, 5-HT and 25C-NBOMe, having high affinity for the receptor, induced ERK activation at the concentration ranges of their Ki values. In contrast, methallyescaline, which had relatively weak affinity for the receptor, failed to induce ERK activation at doses lower than its Ki value.

Endocytic properties of 5-HT_{2A}R agonists

Effects of 5-HT agonists on endocytosis of 5-HT_{2A}R were determined. One compound was selected from each family (25C-NBOME from phenethylamine, 5-BAMT from tryptamine family, and 2-bromo-α-ergocryptine from ergoline family) to examine whether the structural characteristics of the compounds were related to their capacity to induce receptor endocytosis. As shown in Fig. 2A, 5-HT induced endocytosis of 5-HT_{2A}R in a time-dependent manner. All the compounds tested showed similar extent of endocytosis (Fig. 2B), suggesting that the structural features of agonists do not play critical roles in determining their endocytic activities.

Endocytosis of receptors decreases their number on the cell surface, which can be perceived as a mechanism of negative feedback to protect cells from agonistic overstimulation (Sibley and Lefkowitz, 1985). Therefore, blockade of receptor endocytosis is expected to increase their number on the plasma membrane and enhance receptor signaling. In accordance with this expectation, inhibition of receptor endocytosis with co-expression of K44A-dyanmin2, a dominant negative (Fig. 3A) increased ERK activation (Fig. 3B). Dynamin is a critical component involved in the clathrin-mediated and caveolar endocytosis. It cuts the neck of nascent vesicles from the cell membrane, and inhibition of this process by K44A-dynamin2 blocks receptor endocytosis (van der Bliek *et al*., 1993; Kim *et al*., 2001).

DISCUSSION

The SAR assessment revealed that phenethylamine derivatives showed higher binding affinity towards 5-HT_{2A}R than did tryptamine derivatives.

In the phenethylamine group, i) compounds with alkyl or halogen groups at R^1 had affinity to 5-HT_{2A}R higher than those with alkoxy or nitro group; ii) compounds with oxygen-containing group at R^4 showed higher affinity to 5-HT_{2A}R than did their counterparts; and iii) the presence of methoxy groups on

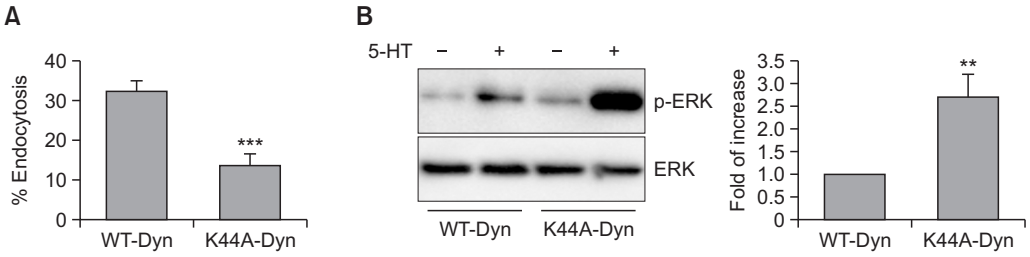


Fig. 3. Effects of 5-HT_{2A}R endocytosis on ERK activation. (A) HEK-293 cells were transfected with 2 μg HA-tagged 5-HT_{2A}R cDNA in pRC/CMV together with 4 μg WT-dynamin2 or K44A-dynamin2 constructs in pCMV5. Cells were treated with 1 μM 5-HT for 15 min. ***p<0.001 compared to the WT-dynamin2 group (n=3). (B) HEK-293 cells were transfected as mentioned above. Cells were treated with 1 μM 5-HT for 5 min. **p<0.01 compared to the WT-dynamin2 group (n=3).

R^5–R^7 exerted strong negative effects on the affinity towards 5-HT_{2A}R.

In the tryptamine group, i) compounds with alkyl groups at R^1 showed lower affinity to 5-HT_{2A}R than did their counterparts; ii) compounds with hydroxyl group at R^2 showed high affinity to 5-HT_{2A}R; iii) substitutions at R^3, regardless of their electron-donating (CH_3, OH, and OMe) or electron-withdrawing (Cl and Br) nature, exerted favorable effects on their affinity towards 5-HT_{2A}R; and iv) allyl groups at R^5 and R^6 exerted strong negative effects on their binding affinity towards 5-HT_{2A}R.

In Table 2, information on the compounds reported in previous publications was collected and analyzed to construct a more precise SAR for tryptamine derivatives. Although ligand affinity for receptors is not significantly affected by the cell types used compared to signal transduction, it should be recognized that these results were obtained in different cellular environments.

Extracellular signal-regulated kinases are key cellular components that control various aspects of cellular functions such as cell proliferation, differentiation, and synaptic plasticity (Brown and Gerfen, 2006; Girault *et al*., 2007). The regulation of ERK through GPCRs is a complicated process, and various signaling components play different roles in ERK activation depending on the GPCR and cell types involved (Beom *et al*., 2004). ERK plays important roles in the pathogenesis, symptomatology, and treatment of depression (Wang and Mao, 2019), which are closely related to the functional roles of 5-HT_{2A}R. For example, ERK levels were altered in the postmortem frontal cortex of patients of mood disorders and schizophrenia (Yuan *et al*., 2010).

During endocytosis, receptor signaling may diminish because the number of receptors that can bind to agonists decreases as the receptors on the plasma membrane move to the cytoplasmic regions (Ferguson *et al*., 1996). Follow-up studies, however, suggested that the main functional role of receptor endocytosis is a restoration of receptor responsiveness rather than a decrement in signaling (Yu *et al*., 1993; Cho *et al*., 2010). However, the basis of endocytosis-induced reduction in signaling or rehabilitation of the function of desensitized receptors is largely unexplored. Intuitively, inhibition of receptor endocytosis would increase receptor signaling when a significant proportion of receptors is endocytosed.

Overall, in this study, a SAR was established for binding affinities of 5-HT_{2A}R agonists of the phenethylamine and tryptamine family members. In addition, ERK activation and receptor endocytosis were evaluated for selected compounds. Since 5-HT_{2A}R is closely related to affective disorders and drug addiction in humans, these findings will provide valuable information for developing therapeutic agents to treat the related diseases.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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