ンパク質／カルシウムイメージング法では，アゴニスト，アンタゴニスト，パーシャルアゴニストなどの薬剤とGPCRとの相互関係は保持されると考えられた．

おわりに

本稿では，主にGiと共役するGPCRに関してキメラGタンパク質を用いてカルシウムイメージングでアッセイする方法について解説した．一方，Gsと共役するGPCRに関しても，G16/sのキメラGタンパク質を作成すれば，同様にカルシウムイメージング法によって機能解析できるが，この場合はもっと簡単にG15やG16を直接GPCRと共発現してもカルシウムイメージング法で解析できる[10]．このように，G16/i，G16を用いることにより，Gi，Gs，Gqと共役する全てのGPCRをカルシウムイメージング法という共通のプラットホーム上で解析ができるようになり，このテクニックはハイスループットスクリーニングなどの技術への応用に適していると考えられる．

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Behavioural Pharmacology

Geissoschizine methyl ether has third-generation antipsychotic-like actions at the dopamine and serotonin receptors

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ABSTRACT

Aripiprazole has made a significant contribution to the treatment of schizophrenia and related disorders. It has improved its safety and tolerability profiles, and these effects have been attributed to its pharmacological profile at the serotonin 5-HT and dopamine D_2 receptors. To discover compounds that have a similar pharmacological profile, we introduced a generic single-cell-based calcium imaging assay that standardizes the readouts from various assays used in previous studies on aripiprazole. In the present assay, the efficacy and potency of known ligands of serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_7 and dopamine D_{2L} receptors were comparable to those found in previous studies using a variety of readouts. The developed assay was also able to reproduce the partial agonist activity, the low intrinsic activity and the selective activation of aripiprazole at the dopamine D_{2L} receptors. Under identical experimental conditions, geissoschizine methyl ether (GM), a plant indole alkaloid, behaved as a partial agonist at the serotonin 5-HT_{1A} receptor, a partial agonist/antagonist at the dopamine D_{2L} receptor and an antagonist at the serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. Interestingly, GM showed a relatively low intrinsic activity and evoked a partial activation response in a subset of cells expressing the dopamine D_{2L} receptor; both of these effects were similarly observed for aripiprazole. Although GM is far less potent at the dopamine receptor than aripiprazole at dopamine D_{2L} receptors (EC_{50} = 4.4 μM for GM *vs.* EC_{50} = 56 nM for aripiprazole), GM and GM derivatives may comprise a new set of candidates for atypical antipsychotics.

1. Introduction

Atypical antipsychotic drugs have revolutionized the treatment of schizophrenia and related disorders. Most approved antipsychotics to date behave as antagonists for the dopamine D_2 and serotonin 5-$HT_{2A/2C}$ receptors. However, an off-base dopamine D_2 receptor blockade in the dorsal striatum and pituitary is thought to cause extrapyramidal symptoms and hyperprolactinemia, respectively (Miyamoto et al., 2005). In contrast, aripiprazole acts as a partial agonist against dopamine D_2 receptors with a 'functionally selective' action, in addition to a partial agonist and antagonist effects at the serotonin 5-HT_{1A} receptors and serotonin 5-HT_{2A} and 5-HT_7 receptors, respectively (Jordan et al., 2007a, 2007b; Shapiro et al., 2003; Urban et al., 2007). Because aripiprazole is effective against the positive and negative symptoms of schizophrenia (Tadori et al., 2005), drugs with similar pharmacological profiles at these receptors are sought to advance the treatment of schizophrenia and related disorders.

For this purpose, we focused on *Yokukansan*, a Japanese *kampo* medicine that has been clinically reported to ameliorate the behavioral and psychological symptoms of dementia (BPSD) in patients with Alzheimer's disease, dementia with Lewy bodies, other forms of senile dementia, borderline personality disorder or schizophrenia (Iwasaki et al., 2005a, 2005b; Miyaoka et al., 2008, 2009; Monji et al., 2009; Shinno et al., 2007, 2008). We hypothesized that *Yokukansan* contains a major substance that affects a variety of serotonin and dopamine receptors.

G protein-coupled serotonin and dopamine receptors differentially stimulate a variety of intracellular signaling pathways. The measurement of cAMP levels is most appropriate for the G protein-coupled receptors linked to $G\alpha_s$ and $G\alpha_i$, whereas assays that measure either the inositol trisphosphate (IP_3) levels or the intracellular calcium levels are optimal for the G protein-coupled receptors linked to $G\alpha_q$. The [^{35}S]GTPγS binding assay is widely used to characterize all types of G protein-coupled receptor functions at one of the earliest receptor-mediated events. However, a previous study showed that aripiprazole, a known D_2 receptor partial agonist, is inactive in the [^{35}S]GTPγS binding assay using both Chinese Hamster Ovary (CHO) cell membranes expressing the cloned human dopamine D_{2L} receptor and CHO-D_{2L} cells, highlighting the limitation of this method for identifying dopamine D_2 receptor partial agonists (Jordan et al., 2007a). Due to this limitation, we introduced a single-cell-based assay that can analyze all types of G protein-coupled receptors on a single platform by

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integrating all of the downstream G protein-coupled receptor-Gα interactions into calcium flux (Ueda et al., 2009).

During screening for antipsychotic agents among the constituents of *Yokukansan*, we found that geissoschizine methyl ether (GM), an indole alkaloid that contains a β-carboline structure (Pengsuparp et al., 2001), inhibited the binding of radioligand to G protein-coupled serotonin receptors in a concentration-dependent manner. In the present study, we investigated the effect of GM on serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors and dopamine D_{2L} receptors using the developed assay system.

2. Materials and methods

2.1. Chemicals

5-Hydroxytryptamine (serotonin; 5-HT), 8-hydroxy-2-(di-n-propylamine)tetralin (8-OH-DPAT), 3-hydroxytyramine (dopamine), quinpirole, *S*(-)-3-(3-hydroxy-phenyl)-N-propylpiperidine (*S*(-)-3-PPP), haloperidol and WAY100635 were purchased from Sigma-Aldrich (St Louis, MO). Aripiprazole was obtained from Toronto Research Chemical, Inc. (TRC; North York, ON, Canada). Geissoschizine methyl ether (GM; $C_{22}H_{26}N_2O_3$) (Fig. 1) was provided from Tsumura Research Laboratories (Tsumura & Co, Ibaraki, Japan). Three different lots of GM were used in the present study, and there were no significant differences in the efficacy and potency on the dopamine and serotonin receptors between the different lots.

2.2. Construction of the Gα proteins and chimeras

A variety of G protein α subunits (Gα) were obtained from a human cell line and mouse brain tissues using reverse transcriptase-polymerase chain reaction (RT-PCR). Human $G\alpha_{16}$ was obtained from HL60 cells, whereas mouse $G\alpha_{15}$ was obtained from mouse blood. The $G\alpha_o$, $G\alpha_{i2}$ and $G\alpha_{i3}$ cDNAs were all cloned from mouse brain tissue. All of the chimeras were constructed by PCR using the human $G\alpha_{16}$ and the mouse-appropriate Gα cDNAs as templates. We constructed $G\alpha_{16}$-based chimeras by replacing the 44-residue C-terminal tail of the $G\alpha_{16}$ with those of $G\alpha_o$ and $G\alpha_{i3}$ ($G_{16/o}$ and $G_{16/i3}$) (Ueda et al., 2009). All full-length α-subunit cDNAs were subcloned into the pcDNA3.1(+) mammalian expression vector (Invitrogen).

2.3. Construction of the serotonin and dopamine receptors

Each open reading frame with a 5′ non-coding sequence of amplified mouse cDNA coding for the serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors and the dopamine D_1 and D_{2Long} (D_{2L}) receptors was subcloned into the pcDNA3.1(+) mammalian expression vector.

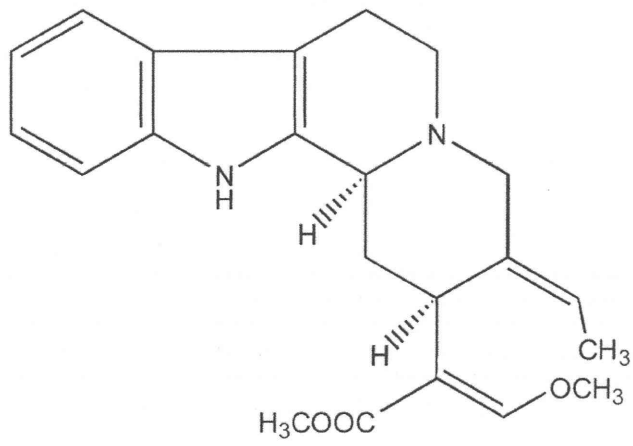

Fig. 1. Chemical structure of geissoschizine methyl ether.

2.4. Transfection of HEK293T cells

Human embryonic kidney 293 T (HEK293T) cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS; v/v) at 37 °C in humidified air with 5% CO_2. For transfection, the cells were seeded onto 100-mm dishes or uncoated glass coverslips in 35-mm chambers. After 24 h at 37 °C, the cells were washed in DMEM and transiently transfected with the Gα subunits and a receptor of interest using Lipofectamine 2000 (Invitrogen). We used $G\alpha_{15}$ and $G\alpha_{16}$ for $G\alpha_s$-coupled serotonin 5-HT_7 and dopamine D_1 receptors, $G_{16/o}$ and $G_{16/i3}$ for $G\alpha_i$-coupled serotonin 5-HT_{1A} and dopamine D_{2L} receptors, or no Gα for $G\alpha_q$-coupled serotonin 5-HT_{2A} and 5-HT_{2C} receptors. The transfection efficiencies were estimated by cotransfection with a GFP reporter plasmid or by immunohistochemistry and were typically >70%.

2.5. Calcium imaging analysis

A single-cell-based reporter system was used to examine the effects of the drugs on the G protein-coupled receptors (Ueda et al., 2009). Transfected cells on glass coverslips were moved 24 h after transfection to an assay chamber and loaded with 5 μM Fura-2/AM (Invitrogen, USA) for 30 min at room temperature. The cells were washed in 500 μl of assay buffer (10 mM HEPES, 130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$ and 1.2 mM $MgCl_2$, pH 7.4) and stimulated with test compounds for 20 s using a bath perfusion system at a flow rate of 5 ml/min. To ensure that the cells were not desensitized as a result of previous ligand applications, a 180- to 420-s interval was maintained between each application of test compounds. The antagonistic effects of the compounds were tested by simultaneous application of the drugs with dopamine, 5-HT or GM for 20 s. We randomly selected Fura-2-loaded cells (100 cells/assay), and the effect of the test compounds on the internal calcium mobilization was measured with the commercially available ARGUS/HisCa system (Hamamatsu Photonics, Japan). The system was set for bottom-up reading with two alternative excitation wavelengths (340 nm and 380 nm) and a 510 nm emission wavelength. Ratios (340 nm/380 nm) were obtained by calculating the fluorescent intensities at 510 nm using the 340 nm and 380 nm excitation wavelengths with the ARGUS/HisCa software v1.65.

For further analysis, we selected cells that exhibited concentration-dependent internal calcium mobilization following the application of compounds (n = 10–15 cells per assay). The cells showing spontaneous and oscillating activities in response to 5-HT or dopamine were omitted. The specificities of all of the compounds to the receptors were confirmed by performing similar assays in non-transfected cells or cells transfected with Gα chimeras only. All of the compounds examined in the present study did not show any significant Ca^{2+} mobilization in non-transfected cells or cells transfected with Gα chimeras.

2.6. Data analysis

A three-parameter logistic equation was fit to the data to calculate the EC_{50}, pEC_{50} and E_{max} values (Prism version 4.0; GraphPad, Inc., San Diego, CA). Data are reported as the mean ± standard error of the mean (S.E.M.) using the traces of Ca^{2+} responses for each cell showing serotonin or dopamine responses in 3 to 5 separate experiments (n = 10–15 cells per experiment). Statistical comparisons were performed using paired *t*-test. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. The present cell-based assay at the serotonin 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A}, 5-HT_7 and the dopamine D_{2L} receptors

We first evaluated all types of serotonin and dopamine receptors of interest in the present assay system. Fig. 2 shows the dose–response curves of known ligands of the serotonin 5-HT_{2A} and 5-HT_{2C} receptors ($G\alpha_q$-coupled receptors) (Fig. 2A), the serotonin 5-HT_{1A} receptor (Fig. 2B), the dopamine D_{2L} receptor (Fig. 2C) ($G\alpha_i$-coupled receptors) and the serotonin 5-HT_7 receptor ($G\alpha_s$-coupled receptors) (Fig. 2D). In Table 1, the results obtained with the present method were compared with the findings from previous studies, and the results show that the pEC_{50} values obtained using the known ligands in the present assay were comparable to those determined from previous assays, such as phosphoinositide (PI) hydrolysis, the cAMP assay and the cell-based ELISA measurements of MAPK activation (Braden et al., 2006; Dunlop et al., 1998; Jordan et al., 2007a, 2007b; Lovenberg et al., 1993; Muntasir et al., 2006; Shapiro et al., 2003; Urban et al., 2007)(Table 1). In the present assay, aripiprazole behaved as a dopamine D_{2L} receptor partial agonist, as did *S*(-)-3-PPP (Fig. 2C), which indicated that the present assay was sufficient to search for dopamine D_2 receptor partial agonists with relatively low intrinsic activity.

3.2. Geissoschizine methyl ether (GM) inhibits the activation of serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors

Previous *in vivo* studies suggested that GM acted as an antagonist for the serotonin 5-HT_{2A} and 5-HT_{2C} receptors (Pengsuparp et al., 2001). The inhibitory effect of GM was next examined on the serotonin 5-HT_{2A} and 5-HT_{2C} receptors, as well as the serotonin 5-HT_{1A} receptor. When 5-HT was used at concentrations of 10 nM (5-HT_{1A}) or 100 nM (5-$HT_{2A/2C}$), GM significantly inhibited the 5-HT-induced responses of the serotonin 5-HT_{2A} and 5-HT_{2C} receptors in a concentration-dependent manner (Fig. 3A). The IC_{50} values were 13 ± 8.6 μM and 8.5 ± 5.2 μM, respectively (Table 2). Similarly, aripiprazole slightly suppressed 5-HT-induced Ca^{2+} mobilization at the serotonin 5-HT_{2A} and 5-HT_{2c} receptors (Fig. 3B). The IC_{50} values were comparable to those of GM (6.2 μM for 5-HT_{2A} and 6.4 μM for 5-HT_{2C}). In contrast, both GM and aripiprazole failed to suppress 5-HT-induced intracellular calcium ($[Ca^{2+}]_i$) mobilization by the serotonin 5-HT_{1A} receptor even at concentrations as high as 40 μM (Fig. 3A and B). In our assay system, spiperone, a serotonin 5-HT_{1A} receptor antagonist, inhibited the response of the serotonin 5-HT_{1A} receptor at 10 nM 5-HT in a concentration-dependent manner (data not shown), which indicated that GM was an antagonist of both the serotonin 5-HT_{2A} and 5-HT_{2C} receptors but not the serotonin 5-HT_{1A} receptor.

We examined whether GM could also act as a partial agonist on the serotonin 5-HT_{2A} and 5-HT_{2C} receptors. It had previously been reported that aripiprazole acted as a partial agonist with an EC_{50} of 48 nM and an intrinsic activity 12.7% that of 5-HT in GF62 cells (Shapiro et al., 2003). At higher concentrations (20 μM), GM caused a slight but significant increase of $[Ca^{2+}]_i$ in cells expressing the serotonin 5-HT_{2A} or 5-HT_{2C} receptors (data not shown). Therefore, GM acted as a partial agonist on these serotonin receptors.

In addition to behaving as an antagonist at the serotonin 5-HT_{2A} and 5-HT_{2C} receptors, it has been suggested that the inhibition of the serotonin 5-HT_7 receptor could be clinically useful for the treatment of positive symptoms in schizophrenia (Galici et al., 2008). Therefore, the effects of GM on the serotonin 5-HT_7 receptor were also determined. GM failed to activate the serotonin 5-HT_7 receptor (data not shown), but it significantly inhibited the 5-HT-induced calcium flux in a concentration-dependent manner (IC_{50} = 610 ± 290 nM) (Fig. 3A). This effect of GM was comparable to that of aripiprazole (IC_{50} = 980 ± 660 nM) (Fig. 3B) (Table 2).

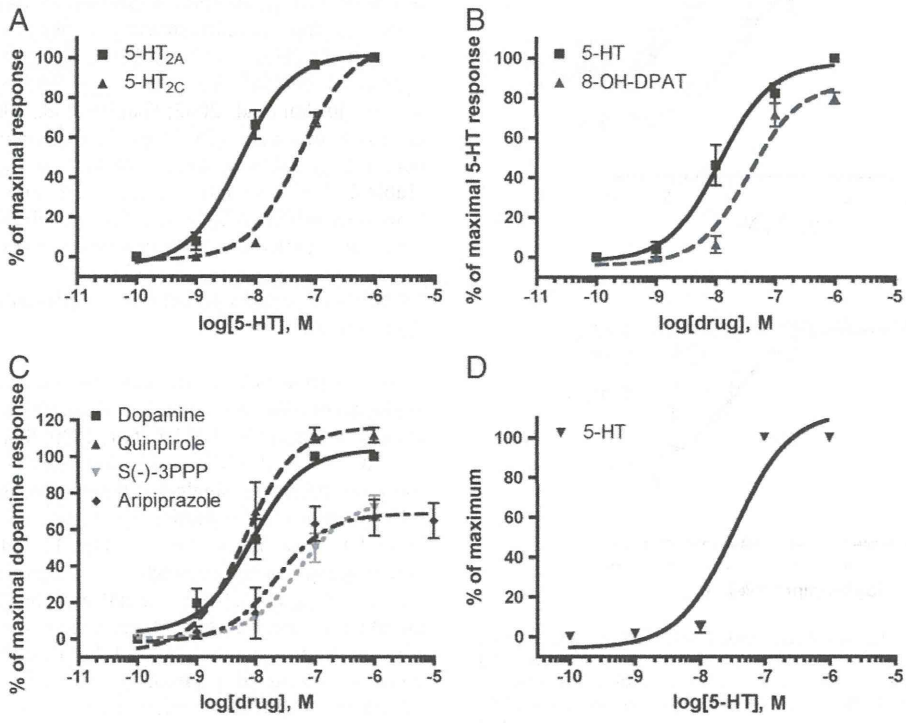

Fig. 2. Concentration–response curves obtained from the present calcium imaging assay for $G\alpha_q$-, $G\alpha_i$- and $G\alpha_s$-coupled serotonin and dopamine receptors. (A) Concentration-dependent ligand-induced stimulation of calcium mobilization by the $G\alpha_q$-coupled serotonin 5-HT_{2A} and 5-HT_{2C} receptors, (B) the $G\alpha_i$-linked serotonin 5-HT_{1A} receptor, (C) the dopamine D_{2L} receptor and (D) the $G\alpha_s$-coupled serotonin 5-HT_7 receptor. Data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing serotonin or dopamine responses in 3 to 5 separate experiments (n = 10–15 cells per experiment). Table 1 compares the data from this study with previous assays.

Table 1
Estimates of pEC_{50} and E_{max} (±S.E.M.) for known ligands at the serotonin 5-HT and dopamine D_2 receptors.

Receptors	Ligands	Previous assays			Present assay	
		pEC_{50}	E_{max} (%)	Assays	pEC_{50}	E_{max} (%)
5-HT_{2A}	5-HT	8.29 ± 0.08	–	PI hydrolysis[a]	8.25 ± 0.08	–
5-HT_{2C}	5-HT	7.17 ± 0.03	–	PI hydrolysis[b]	7.44 ± 0.08	–
5-HT_{1A}	5-HT	8.35	–	cAMP assay[c]	8.01 ± 0.11	–
	8-OH-DPAT	8.70 ± 0.07	98 ± 1	cAMP assay[d]	7.53 ± 0.14	80 ± 3
D_{2L}	Dopamine	7.22 ± 0.09	106.7 ± 3.5	cAMP assay[e]	8.08 ± 0.14	103.6 ± 10.3
		8.06 ± 0.04	100[f]	MAPK assay[g]		
		7.50 ± 0.17	100[h]	[^{3}H] AA release[g]		
	Quinpirole	7.87 ± 0.11	120[f]	MAPK assay[g]	8.19 ± 0.14	116.1 ± 12.3
		7.76 ± 0.04	120[h]	[^{3}H] AA release[g]		
	S(-)-3-PPP	6.28 ± 0.26	20.3 ± 2.9	cAMP assay[e]	7.27 ± 0.15	75.9 ± 11.5
		7.03 ± 0.06	75[f]	MAPK assay[g]		
		7.15 ± 0.08	70[h]	[^{3}H] AA release[g]		
	Aripiprazole	8.36 ± 0.54	10.6 ± 2.0	cAMP assay[e]	7.64 ± 0.33	68.6 ± 13.7
		6.77 ± 0.09	70[f]	MAPK assay[g]		
		8.82 ± 0.11	60[h]	[^{3}H] AA release[g]		
5-HT_7	5-HT	6.81 ± 0.11	–	cAMP assay[i]	7.47 ± 0.1	–

The estimates of pEC_{50} and E_{max} in the present study were determined by the data from the Ca^{2+} responses of each cell showing serotonin or dopamine responses in 3–5 separate experiments.
[a] Braden et al., 2006.
[b] Muntasir et al., 2006.
[c] Shapiro et al., 2003.
[d] Dunlop et al., 1998.
[e] Jordan et al., 2007a, 2007b.
[f] Relative E_{max} values to maximal dopamine response (MAPK assay).
[g] Urban et al., 2007.
[h] Relative E_{max} values to maximal dopamine response ([^{3}H] AA release assay).
[i] Lovenberg et al., 1993.

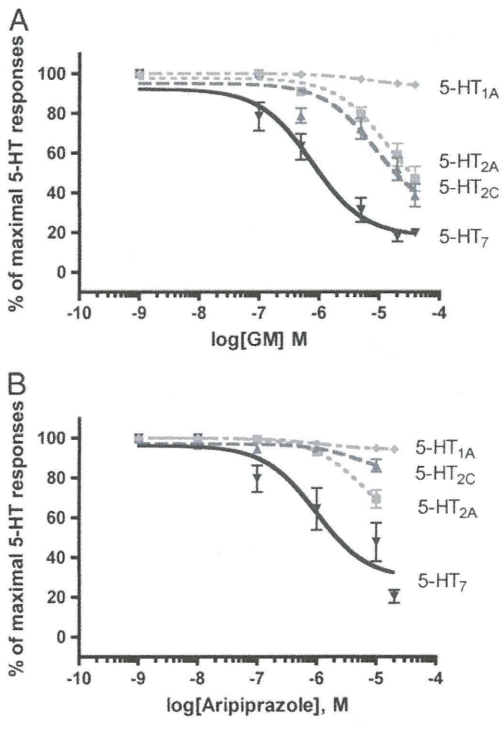

Fig. 3. The antagonistic effect of geissoschizine methyl ether (GM) and aripiprazole at the serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. (A) The effect of GM on 5-HT-stimulated $[Ca^{2+}]_i$ mobilization in HEK293T cells transiently transfected with the indicated serotonin 5-HT receptor. (B) The effect of aripiprazole on 5-HT-stimulated $[Ca^{2+}]_i$ mobilization in HEK293T cells transiently transfected with the indicated serotonin 5-HT receptor. The cells showing a concentration-dependent response to 5-HT were selected and analyzed. All data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing a serotonin response in 3 to 5 separate experiments (n = 10–15 cells per experiment).

3.3. GM activates the serotonin 5-HT_{1A} receptor

A variety of preclinical data suggested that the serotonin 5-HT_{1A} receptor was a therapeutic target for the development of improved antipsychotic drugs (Meltzer, 1999; Millan, 2000). Fig. 4A shows the effect of adding aripiprazole and GM on calcium mobilization by the serotonin 5-HT_{1A} receptor expressed in HEK293T cells transfected with $G_{16/o}$ and $G_{16/i3}$. In our assay, aripiprazole exhibited partial agonist activity (EC_{50} = 210 ± 150 nM; E_{max} = 80 ± 5% of the effect of 1 μM 5-HT on $[Ca^{2+}]_i$ increase) and was consistent with previous studies (Jordan et al., 2002; Shapiro et al., 2003). Similarly, GM stimulated an increase of $[Ca^{2+}]_i$ by the serotonin 5-HT_{1A} receptor with a potent EC_{50} value of 4.6 ± 7 μM and an E_{max} of 85 ± 3% (Fig. 4A) (Table 2). We also confirmed that 5-HT- and the GM-induced activations were inhibited by WAY100635, a selective serotonin 5-HT_{1A} receptor antagonist, in a dose-dependent manner (Fig. 4B and C).

3.4. GM is an unusual partial agonist/antagonist of the dopamine D_{2L} receptor

Aripiprazole partially activated the dopamine D_2 receptor-mediated inhibition of cAMP accumulation, although this action was determined to be system specific (Lawler et al., 1999; Shapiro et al., 2003). Consequently, the effect of GM on the dopamine D_{2L} receptor was analyzed using the presented single-cell-based calcium flux assay in HEK293T cells expressing the dopamine D_{2L} receptor, $G_{16/o}$ and $G_{16/i3}$ in the presence of aripiprazole. As shown in Figs. 1C and 5, aripiprazole acts as a partial agonist on the dopamine D_{2L} receptor in the present assay system. The EC_{50} was 56.0 ± 51.2 nM and the E_{max} was 68.6 ± 13.7% of the maximal dopamine response, both of which fell within the range of values from previous reports (Table 1). Under identical assay conditions, GM displayed a partial agonist activity with an EC_{50} of 4.4 ± 3.6 μM and an intrinsic activity that was 50 ± 15% that of dopamine (Fig. 5) (Table 2). It was noted that GM had a bell-shaped concentration response curve at a higher concentration (100 μM) (Fig. 5), which suggested that strong desensitization and/or antagonism may have occurred.

Table 2
Estimates of pIC_{50}, pEC_{50} and E_{max} (±S.E.M.) for aripiprazole and geissoschizine methyl ether (GM) at the serotonin and dopamine receptors.

Drugs	Receptors						
	5-HT_{2A}	5-HT_{2C}	5-HT_7	5-HT_{1A}		D_{2L}	
	(pIC_{50})	(pIC_{50})	(pIC_{50})	(pEC_{50})	E_{max} (%)	(pEC_{50})	E_{max} (%)
Aripiprazole	5.21 ± 0.76	5.19 ± 1.66	6.01 ± 0.49	6.83 ± 0.39	80 ± 5	7.64 ± 0.33	68.6 ± 13.7
GM	4.87 ± 0.44	5.07 ± 0.41	6.12 ± 0.36	5.34 ± 0.49	85 ± 3	5.36 ± 0.74	50 ± 15

The estimates of pIC50 at the serotonin receptors were derived from the inhibitory responses to 100 nM 5-HT-induced activations.

In addition to the partial agonist activity observed, GM caused an unusual response at the dopamine D_{2L} receptor, similar to aripiprazole. As shown in Fig. 6, the GM-induced $[Ca^{2+}]_i$ activation was only found in a subset of the dopamine-responsive cells and not in all of the cells (Fig. 6A). This partial activation was also observed when aripiprazole was used (data not shown). In addition, the responses obtained from the GM and aripiprazole applications were different from the other dopamine D_2 receptor agonists. For example, activations induced by *S*(-)-3-PPP were observed in all of the dopamine-responsive cells (nearly 100% of the cells), whereas GM activated 53.9% of the dopamine-reactive cells (Fig. 6B). Haloperidol, a potent D_{2L} antagonist, dose-dependently inhibited 10 nM dopamine-induced Ca^{2+} influx (Fig. 6C) in the present assay. Furthermore, it significantly suppressed the number of cells activated by the administration of 10 μM GM (Fig. 6B). These activations were only observed in the dopamine D_{2L} receptor-transfected cells, not in the cells expressing the dopamine D_1 receptor (Fig. 7), indicating that GM selectively activated the dopamine D_{2L} receptor.

We examined the antagonistic effect of GM on the dopamine D_{2L} receptor. As shown in Fig. 8, 20 μM GM conferred a slight but significant inhibition of the dopamine-induced (100 nM) calcium response of the dopamine D_{2L} receptor. These results suggested that GM could be a novel compound that acts as a partial agonist/antagonist on the dopamine D_{2L} receptor with low intrinsic activity and partial activation. These findings raised the possibility that GM functions as a dopamine system stabilizer.

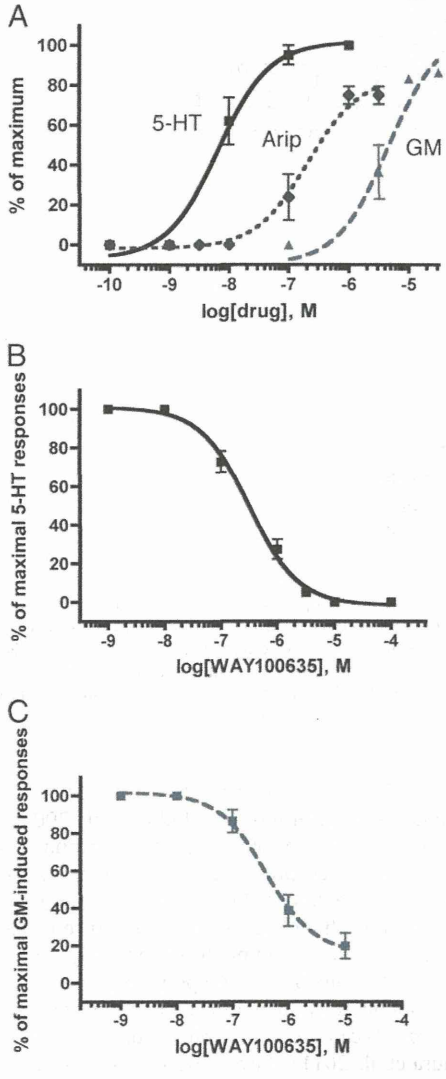

Fig. 4. The effect of geissoschizine methyl ether (GM) and aripiprazole (Arip) on $[Ca^{2+}]_i$ mobilization in HEK293T cells transiently transfected with the serotonin 5-HT_{1A} receptors, $G_{16/o}$ and $G_{16/i3}$. (A) Concentration–response curve of 5-HT, Arip and GM at the serotonin 5-HT_{1A} receptors. (B) The effect of WAY100635 on 5-HT-induced 5-HT_{1A} activation. (C) The effect of WAY100635 on GM-induced 5-HT_{1A} activation. All data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing a serotonin response in 3 to 5 separate experiments (n = 10–15 cells per experiment).

4. Discussion

Atypical antipsychotics act on multiple serotonin and dopamine receptors coupled pleiotropically to various Gα and Gβγ protein subunits, affecting a wide array of signal transduction pathways. In the present study, we introduced a single-cell-based assay system that measures intracellular calcium mobilization to analyze candidate compounds with atypical antipsychotic-like action using $G\alpha_{15/16}$ and their chimeras on a single platform. The efficacy and potency of known ligands generated in the present assay were comparable to those found in previous studies using a variety of readouts (Table 1). Our calcium flux assay could also reproduce the partial agonistic action of aripiprazole with a lower intrinsic activity than *S*(-)-3-PPP at the dopamine D_{2L} receptor (Jordan et al., 2007a, 2007b; Urban et al., 2007), which suggests that the present assay could be useful for rapid screening of a library of compounds for development of new antipsychotic drugs.

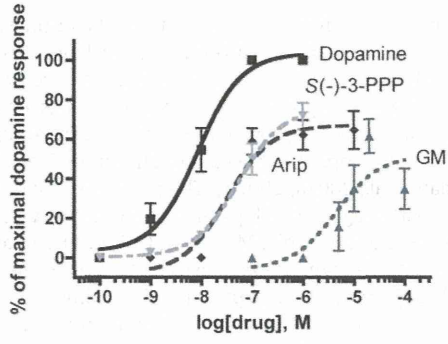

Fig. 5. The effect of dopamine, *S*(-)-3-PPP, aripiprazole (Arip) and geissoschizine methyl ether (GM) on $[Ca^{2+}]_i$ mobilization in HEK293T cells transiently transfected with the dopamine D_{2L} receptors, $G_{16/o}$ and $G_{16/i3}$. All data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing a dopamine response in 3 to 5 separate experiments (n = 10–15 cells per experiment).

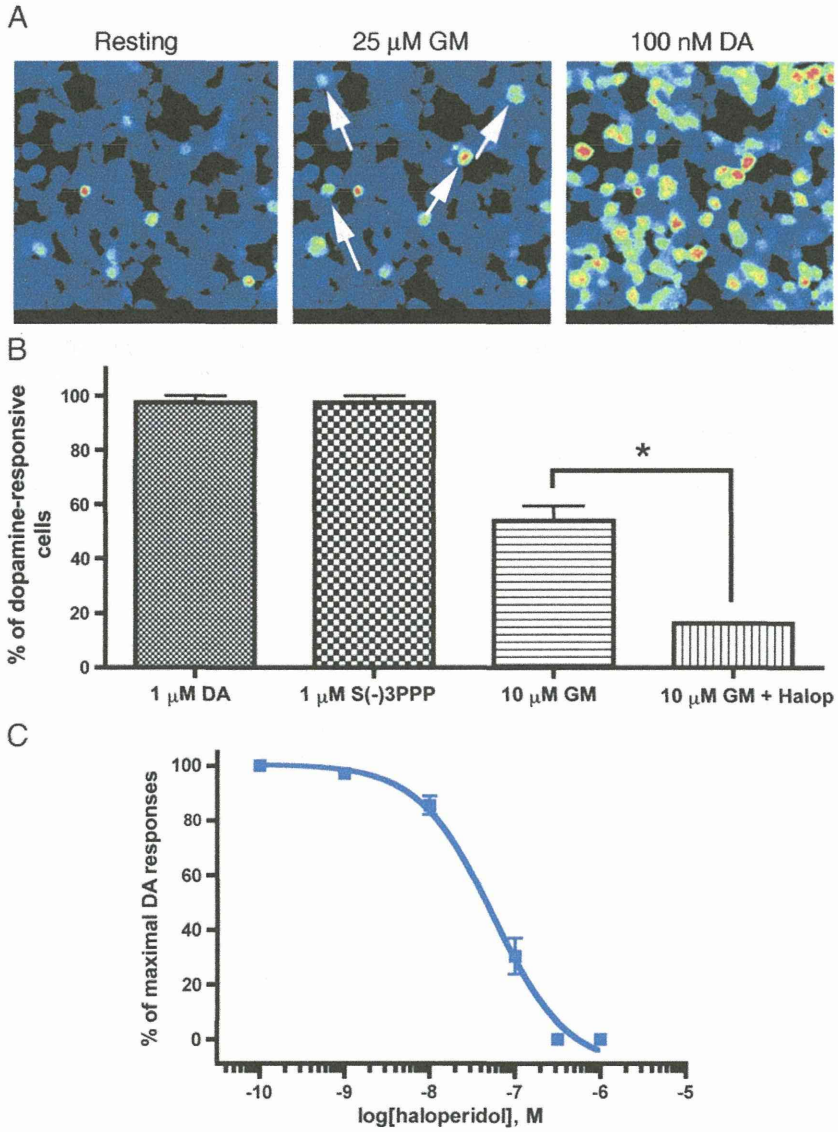

Fig. 6. Partial activations induced by geissoschizine methyl ether (GM) in cells transiently transfected with the dopamine D_{2L} receptor with $G_{16/o}$ and $G_{16/i3}$. (A) Representative images at 1 min after stimulation show that GM caused a partial increase of $[Ca^{2+}]_i$ in a subset of the dopamine-responsive cells (arrows), whereas dopamine (DA) substantially activated a large number of cells. (B) The quantitative analysis showed that *S*(-)-3-PPP activated all of the dopamine-responsive cells (100% of DA-responsive cells), whereas GM partially stimulated these cells (53.9% of DA-responsive cells). The activation induced by GM was significantly suppressed by the simultaneous administration of haloperidol (Halop, 10 μM). (C) Concentration-dependent inhibitory effect of haloperidol on DA-induced Ca^{2+} influx in these cells. All data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing a dopamine response in 3 to 5 separate experiments (n = 10–15 cells per experiment). *P<0.01 *vs.* 10 μM GM.

Dopamine D_2 receptors are important targets for pharmaceuticals used in the management of schizophrenia, Parkinson's disease and drug abuse. Most approved antipsychotic drugs behave as antagonists at the dopamine D_2 receptors, whereas aripiprazole is a dopamine D_2 receptor partial agonist approved as an effective treatment for positive and negative symptoms of schizophrenia and schizoaffective disorder (Jordan et al., 2007a, 2007b). The present study demonstrated that GM is a partial agonist at dopamine D_{2L} receptors with similar intrinsic activity to aripiprazole. Moreover, GM, similar to aripiprazole, partially activated dopamine-responsive cells among the cells transfected with dopamine D_{2L} receptor and Gα chimeras. Because this partial activation was not observed in quinpirole and *S*(-)-3-PPP, GM could share certain characteristics of aripiprazole. However, the molecular mechanism underlying this phenomenon is still unknown. Aripiprazole is postulated to function as a regionally specific modulator of dopamine tone by decreasing high "basal" dopaminergic tone (antagonist) in regions with low dopamine D_2 receptor reserves and increasing low "basal" dopaminergic tone (agonist) in regions with high dopamine D_2 receptor reserves (Tadori et al., 2009). In addition, it has been accepted that aripiprazole has functionally selective actions at dopamine D_2 receptor-mediated signaling pathways (Urban et al., 2007). Thus, the details of GM-evoked partial activation at dopamine D_2 receptors should be addressed in future research.

Atypical antipsychotics commonly exhibit antagonist properties to the serotonin 5-HT_{2A} and 5-HT_{2C} receptors. The present study directly showed that GM has an antagonist activity at the serotonin 5-HT_2 receptors *in vitro*. GM can access the brain through the blood–brain barrier (Imamura et al., 2011). Previously, an *in vivo* study showed that GM reduced the 5-hydroxy-L-tryptophan (*l*-5-HTP) plus clorgyline-induced head switch response thought to be mediated by the serotonin 5-$HT_{2A/2C}$ receptors in a dose-dependent manner (>10 mg/kg i.p.) (Pengsuparp et al., 2001). Although correlations between the *in vitro* and *in vivo* studies across species are confounded by the extensive metabolism of the compound of interest (Wood et al., 2006), it is

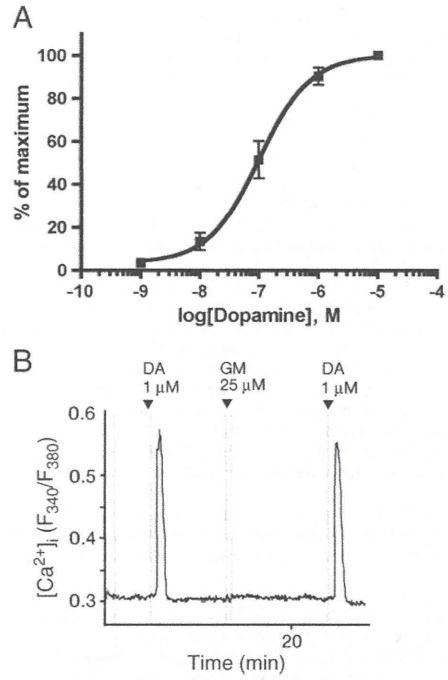

Fig. 7. The results obtained from the cells transiently transfected with the dopamine D_1 receptor with G_{15} and G_{16} as the $G\alpha_s$-coupled receptors. (A) Concentration-dependent dopamine-induced stimulation of $[Ca^{2+}]_i$. All data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing a dopamine response in 3 separate experiments (n = 10–15 cells per experiment). (B) A representative trace showing that GM did not cause any calcium response in dopamine (DA)-responsive cells.

possible that GM is effective in living organisms despite the relatively high IC_{50} values obtained in our *in vitro* assay (serotonin 5-HT_{2A} receptor, 13 μM; serotonin 5-HT_{2C} receptor, 8.5 μM).

The importance of the agonist activity at the serotonin 5-HT_{1A} receptor in antipsychotic drug action has been suggested due to the extensive evidence in rodent models that indicates that the activation of these receptors prevents extrapyramidal symptoms (EPS) induced by dopamine D_2 receptor blockade, favors dopaminergic neurotransmission in the frontal cortex, has a positive influence on mood and opposes NMDA receptor antagonist-induced cognitive and social interaction deficits (Newman-Tancredi, 2010). In this study, we found that GM was a partial agonist at the serotonin 5-HT_{1A} receptor with a relatively high intrinsic activity. It has also been reported that GM has an affinity for [^{3}H]8-OH-DPAT binding in mouse cerebral cortex membranes (Ki of 0.8 μM) and that intraperitoneal administration of GM elicited a hypothermic response (Pengsuparp et al., 2001) primarily due to the activation of the serotonin 5-HT_{1A} receptor (Bill et al., 1991; Martin et al., 1992). Therefore, it is conceivable that GM acts as a functionally mixed serotonin 5-HT_{1A} receptor agonist/serotonin 5-$HT_{2A/2C}$ receptor antagonist *in vitro* and *in vivo*.

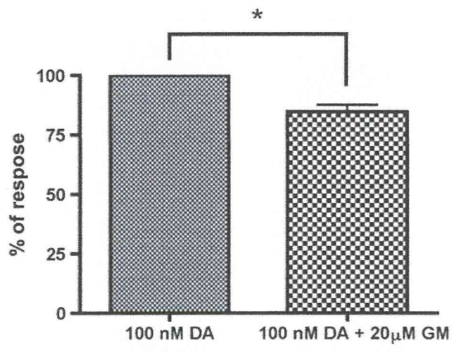

Fig. 8. Geissoschizine methyl ether (GM) partially inhibited the dopamine (DA, 100 nM)-induced $[Ca^{2+}]_i$ response in cells transiently transfected with the dopamine D_{2L} receptor with $G_{16/o}$ and $G_{16/i3}$. All data represent the mean ± S.E.M. from the Ca^{2+} response data for each cell showing a dopamine response in 3 separate experiments (n = 10–15 cells per experiment). *P<0.01 *vs.* 100 nM.

The serotonin 5-HT_7 receptor is a more recently discovered G protein-coupled receptor. As such, the functions and possible clinical relevance of this receptor are not fully understood. Anxiety and schizophrenia models have yielded mixed results with no clear role for the serotonin 5-HT_7 receptor. However, a recent study reported that the blockade of the serotonin 5-HT_7 receptor could be clinically useful for the treatment of the positive symptoms of schizophrenia (Galici et al., 2008). Moreover, there is a considerable amount of evidence that supports a role for the serotonin 5-HT_7 receptor in depression (Abbas et al., 2009; Bonaventure et al., 2007). Therefore, some atypical antipsychotics, including aripiprazole, carry FDA-approved indications for acute mania, bipolar depression, psychotic agitation, and bipolar maintenance, among other indications. Thus, the antagonist activity of GM at the serotonin 5-HT_7 receptor (comparable to aripiprazole) suggested that GM could be an atypical antipsychotic-like compound and may have additional applications in other psychotic disorders.

GM is a plant indole alkaloid and an ingredient of *Chotoko* contained in *Yokukansan* (also referred to as *Yi-gan san*), a traditional Japanese medicine. We previously performed constituent analysis of *Yokukansan* using a competitive binding assay in CHO cell membranes that stably expressed human recombinant serotonin receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_7) and found that compounds with affinities for these serotonin receptors were exclusively contained in *Chotoko* (Terawaki et al., 2010). In support of this finding, the present study demonstrated that GM, a component of *Chotoko*, behaves as a partial agonist at the serotonin 5-HT_{1A} receptor and an antagonist at the serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. In addition, the potency of GM as an antagonist at the serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors was comparable to aripiprazole (Table 2). Moreover, this compound was a partial agonist/antagonist at the dopamine D_{2L} receptor with a relatively low intrinsic activity and partial activation. Thus, the pharmacological profile of GM at the serotonin and dopamine receptors was similar to that of aripiprazole. In agreement with these findings, our preliminary *in vivo* study showed that GM effectively suppressed an aggressive behavior in socially isolated mice (unpublished data). Although GM is far less potent at the dopamine D_{2L} receptors than aripiprazole, further *in vitro* and *in vivo* studies on GM and GM derivatives may contribute to the development of a new treatment for schizophrenia and related disorders. This study also provided new insights into the use of compounds from traditional medicines for the development of novel antipsychotics.

In conclusion, the present study demonstrated that geissoschizine methyl ether is a partial agonist to the serotonin 5-HT_{1A} receptor, a partial agonist/antagonist to the dopamine D_{2L} receptor and an antagonist to the serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. Because the pharmacological profiles closely resemble aripiprazole, geissoschizine methyl ether and derivatives may comprise a new set of candidates for atypical antipsychotics.

Acknowledgments

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解説

脳とくすり

「抑肝散が認知症に有効である」ことを支持する科学的証左

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SUMMARY

アルツハイマー病における神経細胞死は小胞体ストレスに対する応答が悪いためである．抑肝散は小胞体ストレスによる神経細胞死を抑制する．従って，抑肝散はアルツハイマー病による神経細胞死の予防，進行の抑制に効果を発揮する予防薬，治療薬である．

KEY WORDS

抑肝散

アルツハイマー病

神経細胞死

治療薬

はじめに

高齢化に従い認知症の患者は急増している．コリンエステラーゼやNMDA受容体の阻害剤などがその治療薬として開発されているがその効果はおせじにも効果的とはいい難い．一方漢方薬である抑肝散が認知症に有効であるという報告は相次いでいる．はたして，本当に有効なのだろうか？もし有効とすればその科学的裏づけは？本稿では抑肝散が認知症に有効であるという科学的証左を提供する[1]．

我々はアルツハイマー病における神経細胞死は小胞体内に過剰蓄積した不良タンパク質を感知し，その処理にあたる小胞体センサーの異常であることが原因を明らかとしてきた（**図1**）[2-8]．もし抑肝散が認知症に有効だとすれば抑肝散は小胞体ストレスによる神経細胞死を防御するはずである．

I. 抑肝散は小胞体ストレスによる神経細胞死を防御する

先に報告したように培養神経細胞（Neuro2a, N2a）に小胞体ストレス［Thapsigardin（TG）や低酸素刺激（Hypo）］を加えると神経細胞死を引き起こす（**図2 A, B**）．培地に抑肝散を加えると小胞体ストレスによる神経細胞死が救済される．しかしながら非小胞体

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小胞体ストレスとは：細胞内外からさまざまな刺激により小胞体内腔に折り畳み不全の蛋白質がたまる状態

小胞体ストレスが加わると細胞は直ちにストレスから回避するための防御システムを活用化する：Unfolded protein response（UPR）が発動しGRP78を発現，不良タンパク質を処理し細胞を救う．それが不可能な時はCHOPを発現しカスペース4を活性化，細胞死を誘導する．

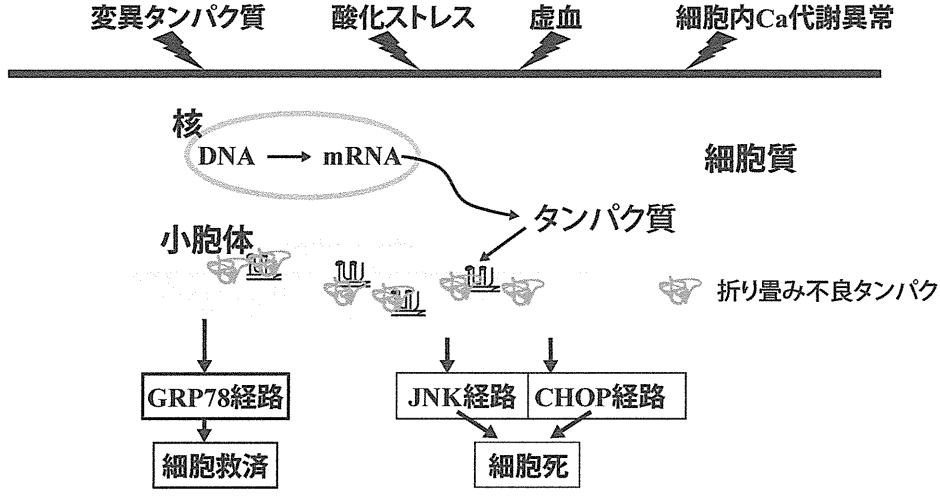

図1　小胞体ストレスとは

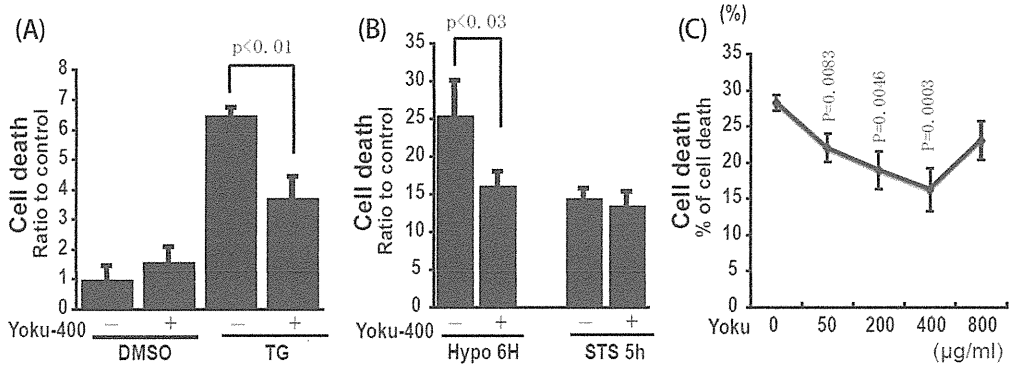

図2　抑肝散は小胞体ストレスによる神経細胞死を抑制する

ストレス（staurosporine; STS）による神経細胞死は抑肝散では救済されない（**図2B**）．しかも，この抑肝散の効果は容量依存性である（**図2C**）．それでは抑肝散を構成する7種の生薬のいずれがこの効果を担うのかを検討した．**図3 A, B**に示すようにマウス，ヒト両者由来の神経細胞の小胞体ストレスによる神経細胞死を抑制するのはセンキュウのみである．

したがって，抑肝散，およびその構成生薬センキュウは小胞体ストレスによる神経細胞死を抑制することが明らかとなった．小胞体ストレスセンサーに感知された小胞体ストレスは下流のカスケードに伝えられ，GRP78発現が優位の時は細胞生存へ，CHOP-カスペース4系が優位の時は細胞死へと神経細胞は誘導される．もし抑肝散，センキュウが小胞体ストレスに対し

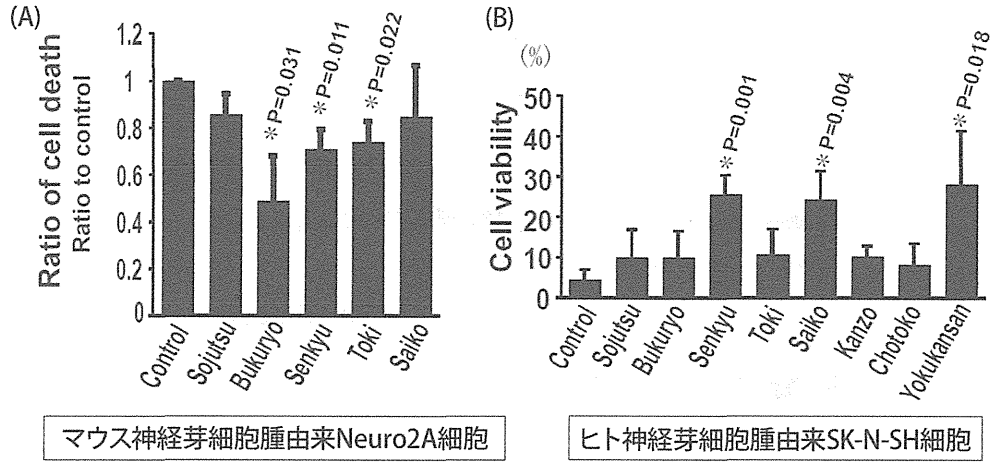

図3　ヒト，マウス由来の神経細胞の両者において抑肝散構成生薬センキュウが小胞体ストレス負荷による神経細胞死を抑制する

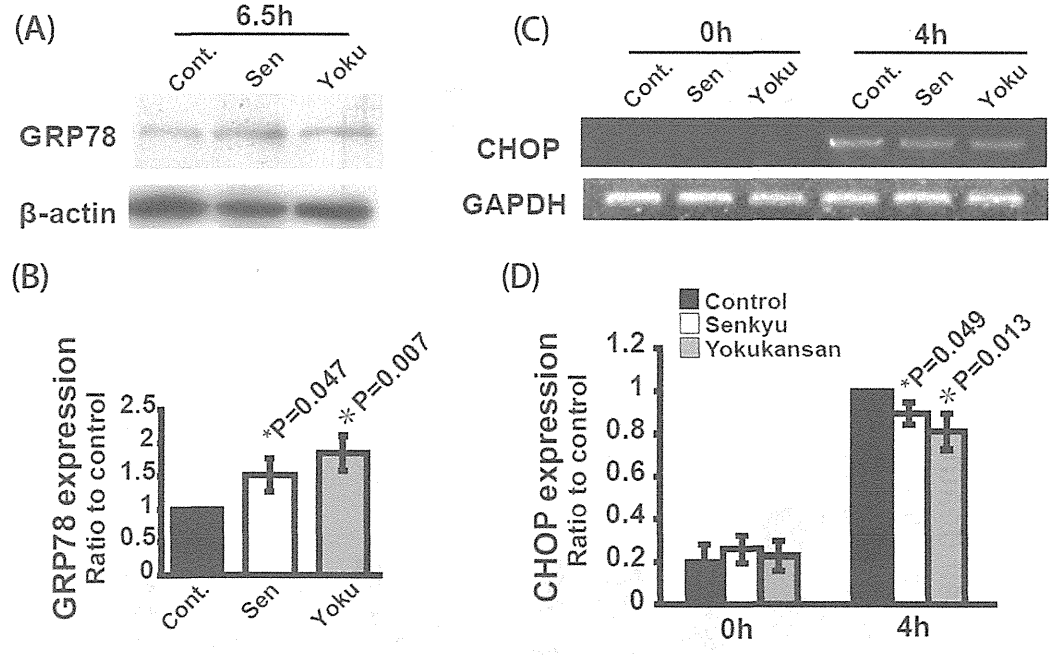

図4　抑肝散，センキュウは小胞体ストレスから細胞死を守るGRP78発現を増強し，細胞死を促進するCHOP発現を抑制する

防御的に働くならばGRP発現の増加に，CHOP-カスペース4の抑制に働かねばならない（図1）．この点についても検討を加えた．

II. 抑肝散，センキュウの小胞体ストレスの下流のカスケードに対する効果

図4 A-Dに示すように抑肝散，センキュウは通常よりもGRP78発現を増強し，CHOP発現を抑制する．

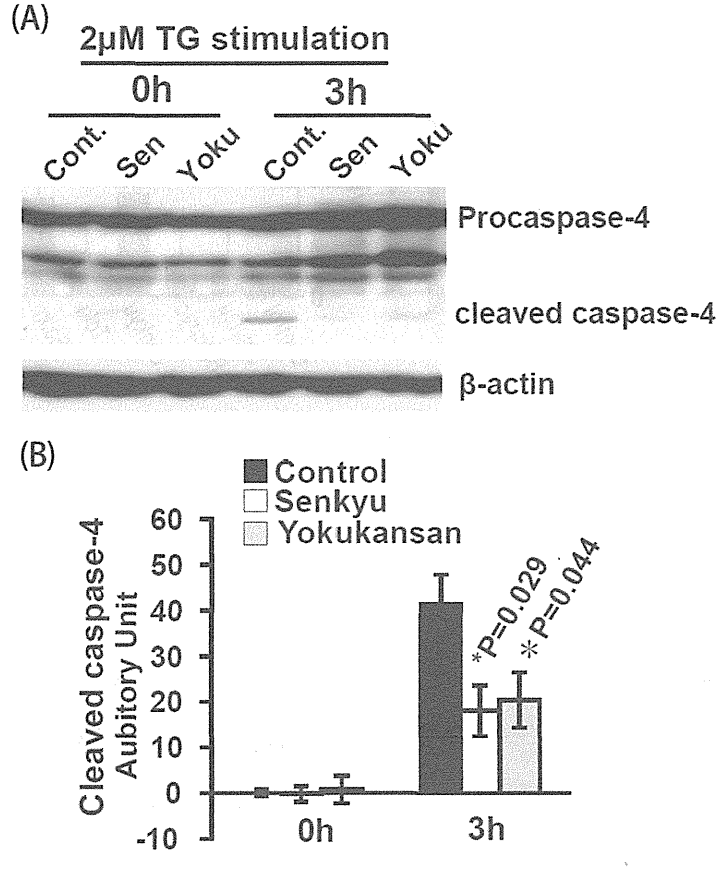

図5　抑肝散，センキュウはカスペース4の活性化を抑制する

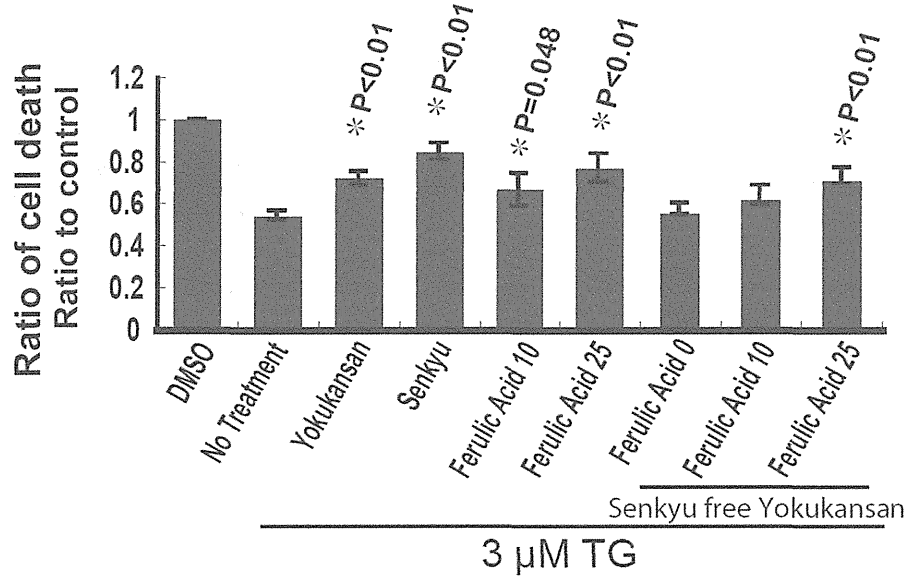

図6　センキュウに含まれるFerulic acidが小胞体ストレスによる神経細胞死を抑制する

さらにこの両者はカスペース4の活性化を阻害する（**図5**）．これらの結果は抑肝散，センキュウは小胞体ストレスセンサー機能をより強めることにより神経細胞死を防いでいることがわかる．それではセンキュウに含まれるどの因子がこの効果を担うかが次の問題となる．

III. センキュウに含まれるフェルラ酸が小胞体ストレスによる神経細胞死を抑える

センキュウに含まれる各種因子を網羅解析した結果フェルラ酸が小胞体ストレスによる神経細胞死を上に述べた細胞防御カスケードを活性化し，細胞死促進カスケードを抑制することにより神経細胞死を救済することがわかった（**図6**）．

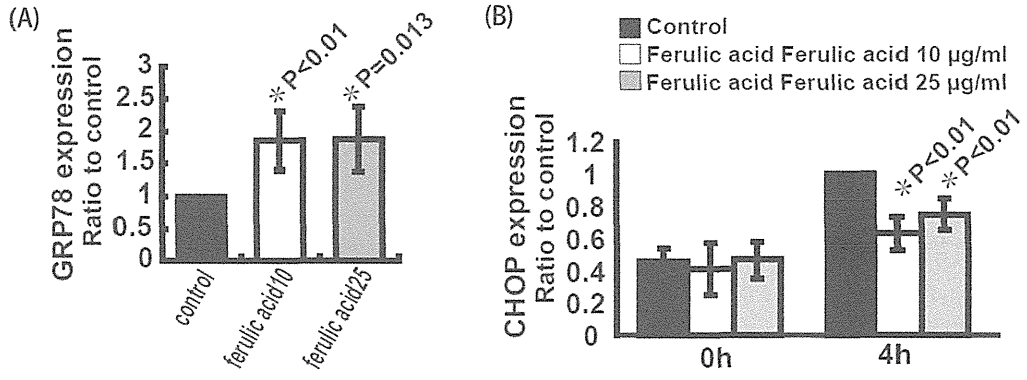

図7　Ferulic acid は細胞死を救済する grp78 発現を高め 細胞死を誘導する CHOP 発現を抑制する

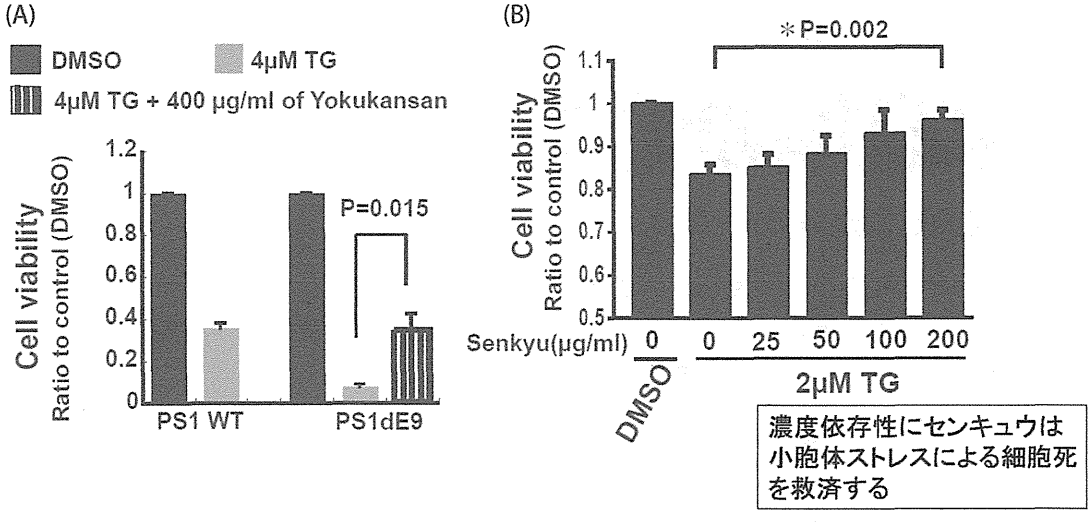

図8　センキュウは PS1 変異体発現細胞の小胞体ストレスに対する脆弱性を救済する

IV. アルツハイマー病による認知症にも抑肝散は有効である

家族性アルツハイマー病の原因遺伝子プレセニリン1の変異（dE9）を発現している神経細胞は小胞体ストレスが加えられると正常細胞よりもより早期に神経細胞死に至る（**図8A**）．抑肝散はこの神経細胞死も抑制し，しかもセンキュウは容量依存性に作用する（**図8B**）．

以上の結果より抑肝散は加齢による神経細胞死の予防として，アルツハイマー病による神経細胞死の進行抑制として科学的証左を有する予防薬，治療薬であると結論できる．

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解説

脳とくすり

統合失調症に有効な抑肝散成分の解析

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SUMMARY

認知症に伴う行動心理学的症状（BPSD）の治療には非定型抗精神病薬が効果を示すことが知られている．また，抑肝散もBPSDに対して効果を示すことが報告されていることから，抑肝散に含まれる天然アルカロイド成分の中で非定型抗精神病薬と類似した作用を示す単体の薬物の探索を行うことができれば，統合失調症に有効な新しい薬剤を開発することが可能になると考えられる．そこで非定型抗精神病薬と類似した成分が抑肝散に含有されている可能性について検討した．その候補成分として抑肝散の構成生薬の釣藤鉤のインドールアルカロイド成分に着目し，中枢神経系に発現しているセロトニン受容体のサブタイプ（1A，2A，2C，7），およびドーパミン受容体（D1，D2）に対してどのように作用するかを細胞内 Ca^{2+} イメージング法を用いて解析した．その結果，この成分は第三世代の非定型抗精神病薬アリピプラゾールと類似した特徴を有していることを見出した．

KEY WORDS

ガイソシジンメチルエーテル
アリピプラゾール
セロトニン受容体
ドーパミン受容体
パーシャルアゴニスト

はじめに

認知症に伴う行動心理学的症状（BPSD）の治療に対して抑肝散が効果を示すことが報告されている．BPSDに対して抑肝散は服用後比較的早期に効果があることから，少なくともこの効果には神経伝達機構が直接関与している可能性が高い[1-7]．一方，BPSDの治療にはリスペリドンなどの非定型抗精神病薬が効果的であることが知られている．これらの事実から考え合わせると，非定型抗精神病薬と類似した成分が抑肝散の中にも含有されている可能性があり，抑肝散の構成成分の中に統合失調症に有効な成分が含まれている可能性がある．非定型抗精神病薬の作用機序としてドーパミン D_2 受容体以外にも多種類のセロトニン受容体サブタイプに同時に作用することが知られている[8-12]．そこで本稿では抑肝散の中のアルカロイド成分が，ドーパミン受容体や各種セロトニン受容体サブタイプに対してどの様な影響を与えるか解析した結果を報告する[13]．

I. 細胞内 Ca^{2+} イメージング法の開発

抑肝散やそのアルカロイド成分が，中枢神経系に発現しているタイプのセロトニン受容体（1A，2A，2C，7），さらにドーパミン受容体（D1，D2）に対

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してどの様に作用するか検討するために，まずは細胞内 Ca^{2+} イメージング法の開発を行った．これらのセロトニン受容体はそれぞれ異なるG蛋白と共役するため，セカンドメッセンジャーも異なり，単純にHEK293細胞に各タイプのセロトニン受容体，ドーパミン受容体を発現させただけでは，Ca^{2+} イメージング法で反応が得られない．この点を解消するため，それぞれのタイプのセロトニン受容体，ドーパミン受容体と選択的に共役するGα蛋白とGα16蛋白でキメラ蛋白を作成し，すべての種類のGタンパク質共役型セロトニン受容体またはドーパミン受容体のシグナル伝達をPI turnover系に集約することによりこれら受容体の活性測定に非常に有効な Ca^{2+} イメージング法を確立した[13, 14]．

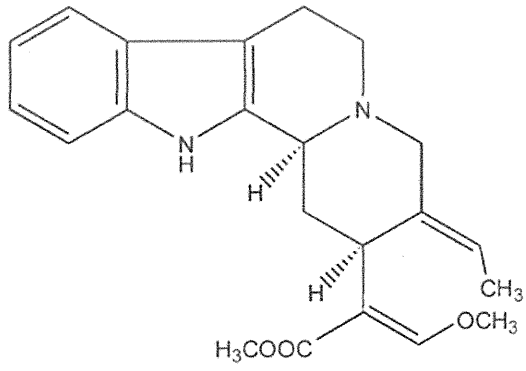

図1　ガイソシジンメチルエーテルの化学構造

II. セロトニン受容体に対するガイソシジンメチルエーテルの作用

まず初めに，さまざまな抑肝散含有成分を検討した結果，抑肝散含有アルカロイドの一成分であるガイソシジンメチルエーテル（**図1**）がセロトニン受容体に影響を与えることが分かった．このアルカロイドは $5\text{-}HT_{1A}$ 受容体，$5\text{-}HT_{2A}$ 受容体，$5\text{-}HT_{2C}$ 受容体，$5\text{-}HT_7$ 受容体にそれぞれ作用するが，$5\text{-}HT_{1A}$ 受容体に対しては，パーシャルアゴニストとして働くことを見出した（**図2**）．$5\text{-}HT_{2A}$ 受容体に対しては，主にアンタゴニストとして働き，$5\text{-}HT_{2C}$ 受容体や $5\text{-}HT_7$ 受容体に対してもアンタゴニストとして働くことを明らかにした（**図3**）．

III. ドーパミン受容体に対するガイソシジンメチルエーテルの作用

さらに著者らは，ガイソシジンメチルエーテルがドーパミン受容体にどのような影響を与えるのか検討を加えた．ガイソシジンメチルエーテルの D_2 受容

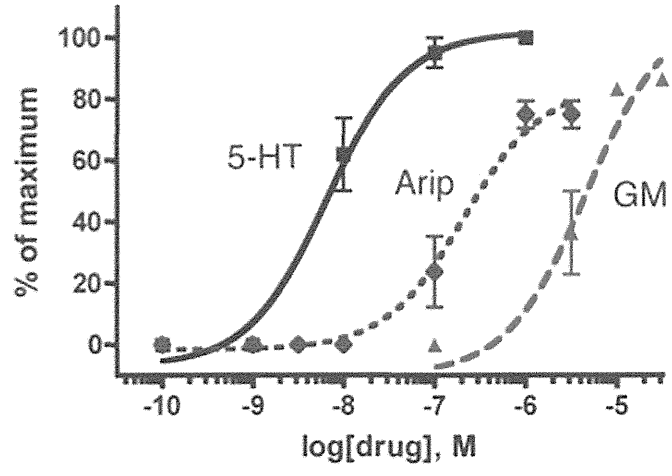

図2　セロトニン（5-HT），アリピプラゾール（Arip），ガイソシジンメチルエーテル（GM）の $5\text{-}HT_{1A}$ 受容体に対する応答性を調べた濃度依存曲線

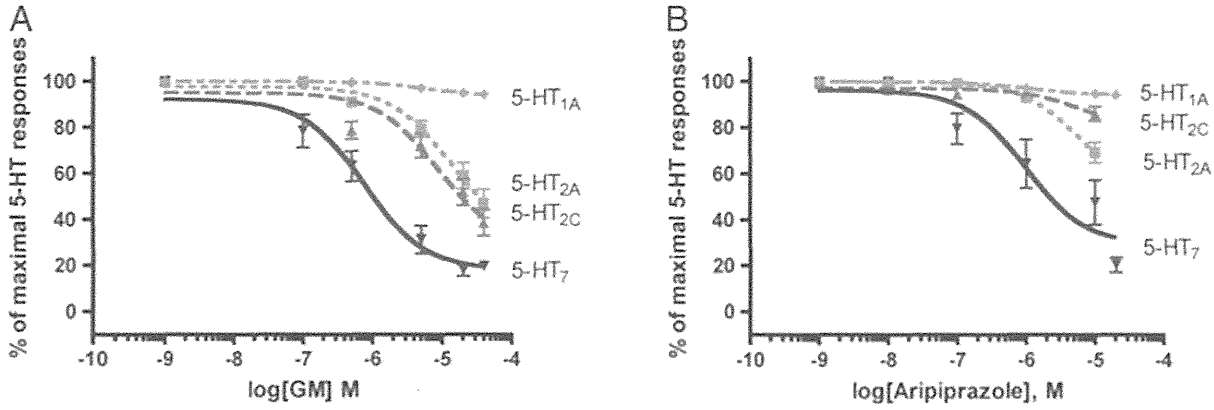

図3　(A) ガイソシジンメチルエーテル (GM), (B) アリピプラゾール (Arip) は 5-HT_{2A}, 5-HT_{2C}, 5-HT_7 受容体に対してアンタゴニスト作用を示した

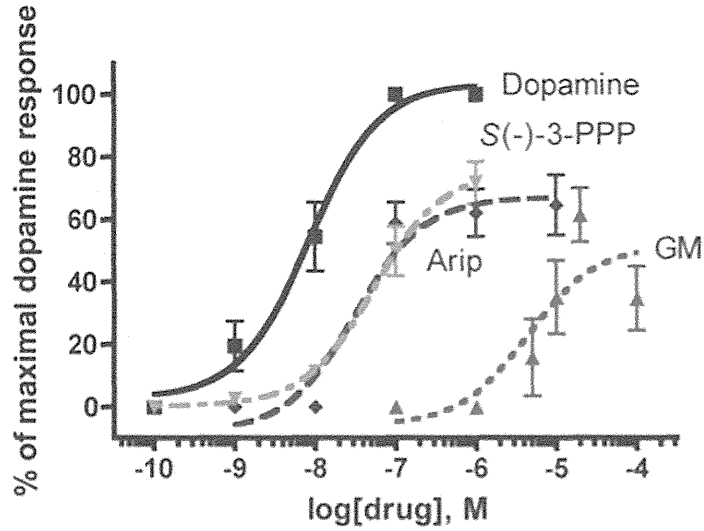

図4　ドーパミン (Dopamine), S(-)-3PPP (D_2 パーシャルアゴニスト), アリピプラゾール (D_2 パーシャルアゴニスト) (Arip), ガイソシジンメチルエーテル (GM) の D_2 受容体に対する応答性を調べた濃度依存曲線

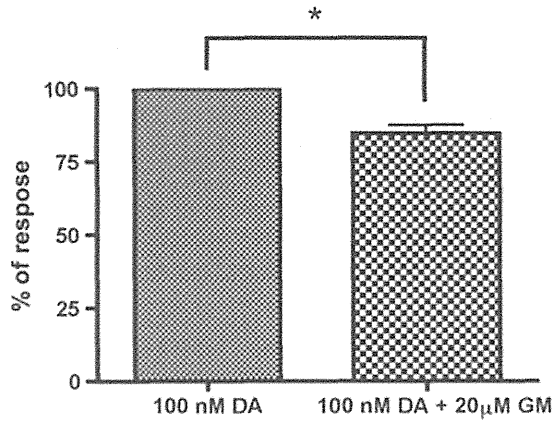

図5　D_2 受容体に対して 100 nM のドーパミン (DA) が示す応答をガイソシジンメチルエーテル (GM) が部分的に阻害した

体に対する応答性を調べた濃度依存曲線から，S(-)-3PPPやアリピプラゾールに比べると親和性は下がるが，ガイソシジンメチルエーテルがD_2受容体のパーシャルアゴニストであることを見出した（pEC_{50}：5.36±0.74，E_{max}：50±15%）（**図4**）．

また，D_2受容体に対して100 nMのドーパミンが示す応答を指標にガイソシジンメチルエーテルがどのような反応を示すか検討したところ，D_2受容体に対してパーシャルアンタゴニストとして働くことも明らかとなった（**図5**）．さらに，D_1受容体についても検討を行ったが，ガイソシジンメチルエーテルの添加ではCa^{2+}イメージングはD_1受容体には全く応答しなかった．

おわりに

抑肝散含有アルカロイドの一成分であるガイソシジンメチルエーテルはセロトニン受容体5-HT_{1A}に対してはパーシャルアゴニストとして，5-$HT_{2A,\ 2C}$，5-HT_7受容体にはアンタゴニストとして作用することを示した．さらにドーパミン受容体での検討では，D_2受容体に対してはパーシャルアゴニストとして作用し，D_1受容体に対しては影響を与えなかった．これらのセロトニン受容体に対するプロフィールとドーパミン受容体に対する結果とを照らし合わせると，ガイソシジンメチルエーテルはD_2受容体パーシャルアゴニストとしての親和性はあまり高くないが，非定型抗精神病薬のアリピプラゾールと類似した薬理学特性を有しており，ガイソシジンメチルエーテルをリード化合物とした新しい抗精神病薬の開発にも繋がる可能性が十分に考えられ，今後の進展が期待される．

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解説

脳とくすり

ストレス応答に対する抑肝散の効果

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SUMMARY

慢性的なストレス負荷はうつ病発症の最大の原因として知られている．これは，繰り返し受ける強いストレスにより視床下部－下垂体－副腎軸（HPA axis）が過剰に反応し続けることでうつ病発症にいたると考えられているが，その分子メカニズムは全くといっていいほど解明されていないのが現状である．

そこで著者らは，まず始めに慢性ストレス負荷マウスを作製し，うつ病態のひとつのモデルマウスであることを確認した．このマウスでは，慢性ストレス特異的に引き起こされる脳内の反応経路が存在した．線維束特異的に反応するこの経路では，グリア細胞のひとつであるオリゴデンドロサイト特異的にSGK1（Serum/glucocorticoid regulated kinase 1）の発現上昇および活性化が引き起こされていた．著者らはこのカスケードへの抑肝散中の有効成分の探索を行ったので報告する．

KEY WORDS

慢性ストレス
SGK1
うつ病
オリゴデンドロサイト

はじめに

認知症に伴う行動心理学的症状（BPSD）の治療に対して抑肝散が効果を示すことはこれまで数多く報告されている[1-7]．さらにこのBPSDの治療には非定型抗精神病薬だけでなく，抗うつ薬も使用されている．これらの事実は，抗うつ薬と類似した成分が抑肝散の中にも含有されている可能性を示しており，抑肝散の構成成分の中にうつ病に有効な成分が含まれている可能性が十分に考えられる．

そこで本稿では，うつ病に効果的な抑肝散成分の探索・同定を目標とした，動物モデルの作製と評価を中心に報告する[8]．

I. 慢性ストレス負荷マウスの作製

著者らはうつ病の病態モデルとして水浸拘束法による慢性的なストレス負荷マウスの作製を試みた．このマウスでは，恒常的に血中のコルチコステロン量が増加しており，尾懸垂法などの行動解析による無気力レベルの上昇や海馬歯状回における神経新生レベルの低下など，うつ病態を示していると考えられ，うつ病の一つの病態モデルであると考えた（**図1**）．

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