生 物 試 験 部 門

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本部門では，和漢薬の新しい薬効評価法を確立するための研究，それにより和漢薬作用を定量的に評価すると共にその作用本体を追究する研究，それらの作用機序を明らかにするための研究を行っている。

本年の主な研究課題と成果は下記の通りである。

Ⅰ．和漢薬の新しい薬効評価法を確立するための基礎研究

1）多発性脳梗塞のモデルとして有用な両側内頚動脈永久結紮ラットにみられる空間学習行動障害は標準薬としてのアルツハイマー病治療薬 tacrine によって著明に改善されることを認めた。

2）当帰の成分やベトナム薬用人参のサポニン成分 Majonoside R2 が長期隔離飼育マウスの短縮したペントバルビタール誘発睡眠を対照群レベルまで回復させること，その作用に $GABA_A$ 受容体機構が連関していることを明らかにした。

Ⅱ．中枢作用薬の神経薬理学的研究

長期隔離ストレスによってマウスのペントバルビタール睡眠時間が短縮するが，その作用には中枢性のホルモン，ニューロステロイドおよびベンゾジアゼピン様物質が関与することを明らかにし，その詳細を研究している。

Ⅲ．遺伝子発現変化を指標とした薬物作用機序の解明と和漢薬作用

両側内頚動脈永久結紮ラットの脳内で誘導される遺伝子を differential display 法により単離し，構造の解析とその意義について検討している。

Ⅳ．新規リード化合物の検索を目指した各種民族薬の薬理作用の解明

1）タイ薬用植物 *Hunteria zeylanica* 葉の主成分 corymine はストリキニーネやピクロトキシンで起こしたマウスの痙攣を増強するが，その作用機序の研究において，アフリカツメガエル卵母細胞に発現したグリシン受容体を介したクロライド電流応答を抑制することを明らかにした。

2）タイ産植物 *Mitragyna speciosa* 葉の主成分 mitragynine は圧刺激に応ずる痛み反応系において，脊髄及び脊髄以上のレベルにおいて μ（μ1 含む），δ および κ オピオイド受容体を介して鎮痛作用を発揮することを明らかにした。

Ⅰ．和漢薬の新しい薬効評価法の確立のための基礎研究

1）学習障害モデル動物としての両側総頚動脈永久結紮（2VO）ラットを用いた各種薬物作用に関する研究

1-1） Candidates for cognitive enhancer extracted from medicinal plants: paeoniflorin and tetramethylpyrazine :

A traditional Chinese medicine, Shimotsu-to, con-

sisting of four herbs: Japanese angelica root, cnidium rhizome, peony root and rehmannia root, has been reported to improve spatial working memory in rats.

The present results show that rats with permanent occlusion of bilateval common carotide arteries (2VO) exhibited severe deficit in the learning of new information on the 8-arm radial maze task, and those reported on the memory loss in normal aged humans and dementia patients. In this study, we found that daily administration of tetramethylpyrazine (TMP) from the third day after permanent 2VO significantly improved this learning deficit. A number of studies using ischemic models have demonstrated that chemicals produced beneficial effects when administered before the ischemic operation, and only a few compounds were reported to have effects after ischemia insult. Thus, the efficacious treatment with TMP 3 days after permanent 2VO suggests the potential therapeutic avail-ability of TMP for treatment of dementia derived from cerebral hypoperfusion and/or cerebral ischemia.

In our previous study, we demonstrated that the permanent 2VO rats could keep the newly acquired information during only a very short period (no more than 3 min) and that such limited ability might contribute to the learning impairment caused by permanent 2VO. Here, although the effect was not statistically significant, TMP administration tended to decrease the number of errors elevated by the 3-min delay interposition. Thus the ameliorating effect of TMP on the learning deficits of permanent 2VO rats may be partly due to the improvement of the process for consolidating newly acquired information.

It is of interest to note that TMP not only improved the cognitive impairments caused by permanent 2VO, but also reversed the scopolamine-induced amnesia. The memory impairment is known to be a cardinal symptom of Alzheimer's disease (AD), and scopolamine reportedly produces the memory impairment similar to AD [7,22,25]. The present results seem to be strong evidence in support of the idea that TMP might have some beneficial effects on cognitive impairment in clinical cases.

The present results indicate that Paeoniflorin and TMP extracted from peony root and cnidium rhizome, respectively, are candidates for cognitive enhancer.

1-2) Tacrine Improves Working Memory Deficit Caused by Permanent Occlusion of Bilateral Common Carotid Arteries in Rats:

Effect of tacrine, a cholinesterase inhibitor, on spatial acquisition deficit caused by permanent occlusion of bilateral common carotid arteries (2VO) was examined by using the conventional 8-arm and the 4-arm baited radial maze tasks in rats. Daily administration of tacrine (0.1 and 0.3 mg/kg, i.p.) 1 month after 2VO operation significantly improved the impaired spatial acquisition in the conventional maze task (Fig. 1). This treatment also ameliorated the 2VO-induced working but not reference memory deficit in the 4-arm baited radial

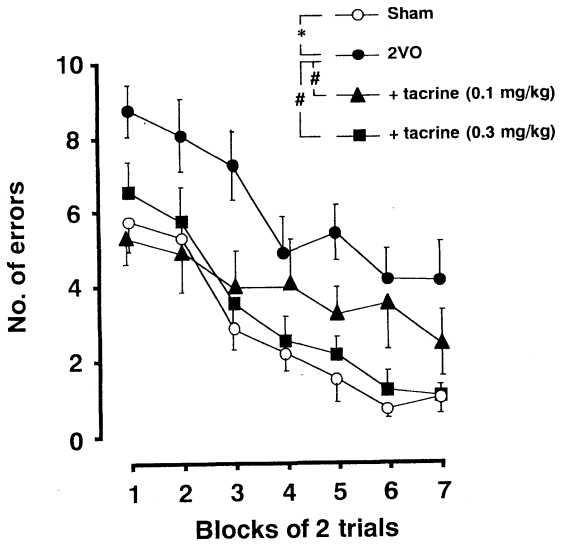

Fig. 1 The effect of tacrine on permanent 2VO-induced spatial acquisition deficit in the conventional 8-arm radial arm maze task. One month after permanent 2VO operation, the daily administration of tacrine was strated. Thirty minutes before each trial, 2VO rats were injected i.p. with saline (closed circles, n=11) or tacrine (closed triangles: 0.1 mg/kg, n=10; closed squares: 0.3 mg/kg, n=11), and sham-operated rats (open circles, n=10) were administered saline alone. Each data point represents the means±S.E.M. of two trials. *P<0.05, compared with the sham group. #P<0.05, compared with the saline-treated 2VO group.

maze task. These results suggest that tacrine improvement of working memory deficit in the 2VO rats is due to stimulation of central cholinergic systems.

2）学習障害モデル動物中で発現変化する遺伝子の検索

Changes of the gene expression in rat with permanent occlusion of bilateral common carotid arteries:

We have been reported that the rat with permanent occlusion of bilateral common carotid arteries (2VO) is a useful model for ischemic disorders, especially in cognitive deficits and neuronal damages. To identify physiological factors changing with the 2VO treatment, differential display method was carried out. Male Wistar rats, 13 weeks old, were 2VO operated. The learning and memory performance were examined using an eight-arm radial maze task. Sixteen weeks after the 2VO, the brain RNA was isolated from the rat with cognitive deficits and sham-operated rat without the deficits. RT/PCR was carried out using oligo(dT) primer and optinal 10 mer primer. We isolated 8 clones showing the differential expression. The expression of five clones was enhanced by 2VO treatment. The molecular size was estimated in 770, 490, 400, 380 and 160 bp, although that may be partial sequences. The sequence of the 490 bp factor was determined (487bp) and was highly homologous (>90 %) with rat K^+ channel RHK1 and RCK4. Three clones of the molecular size, which decreased by 2VO treatment, was estimated in 780, 710 and 530. The 530bp factor was revealed a novel sequence (homology< 30 %) with 533 bp (Table. 1). Other 6 clones are also analyzing, and functional siginificances are studying by in situ hybridization using the digoxigenin-labeled probes.

3）モデル動物を用いた抗痴呆薬の検索

GTS-21, a Nicotinic Agonist, Protects against Neocortical Neuronal Cell Loss Induced by the Nucleus Basalis Magnocellularis Lesion in Rats:

Effect of subchronically administered GTS-21 [3-(2,4-dimethoxybenzylidene)-anabaseine dihydrochloride], a selective nicotinic agonist, on neuronal cell loss caused by nucleus basalis magnocellularis (nBM) lesion was studied in rats. After 2 weeks of bilateral nBM excitotoxic lesion, GTS-21 was orally administered once daily for 20 weeks. Neuronal cell loss was observed in layers II-III of the parietal cortex in the lesioned control rats. GTS-21 significandly attenuated the neuron loss in these layers. These results suggest that GTS-21 exhibits a protective action against the neuronal cell death in the parietal cortex and may have a beneficial effect on enurodegenerative disorders such as na Alzheimer-type disease.

4）興奮性アミノ酸受容体の学習行動への関与の解明

4-1） NMDA antagonists potentiate scopolamine-induced amnesic effect:

The effects of *N*-methyl-D-aspartate NMDA receptor antagonists on scopolamine-induced amnesia and on delay-interposed short-term memory perfomance were investigated using an 8-arm radial maze in rats. Scopolamine, a muscarinic antagonist, deteriorated the radial maze performance, while MK-801, an NMDA receptor channel blocker and CGS-19755, a competitive NMDA receptor antagonist, showed no obstruction to the spatial cognition in the non-delayed maze task. MK-801 (0.01-0.03 mg/kg,i.v.) and CGS-19755 (1-10 mg/kg, i.v.) significantly augmented scopolamine-induced deficit in the non-delayed maze task and impaired the short-term memory in the 5-min delay-interposed task. These results suggest that NMDA antagonists have a negative action on short-term memory and that

Table 1　これまでに単離した両側総頚動脈永久結紮後4カ月の時点で発現変化する遺伝子

	塩基数 (bp)	vector	増減	相同性	accession NO.
vof-16	789	pBlue	↑	新規	AB006880～1
vof-31	523	pGEM	↓	新規	AB006882～3
vof-43	487	pGEM	↑	K channel (90%)	未登録

the interaction between the NMDA and the central muscarinic system plays a role in modulating the cognitive function.

4-2) NMDA but Not AMPA Receptor Antagonists Impair the Delay-Interposed Radial Maze Performance of Rats:

The effects of the *N*-methyl-D-aspartate (NMDA) receptor antagonists CGS19755 and MK801 and the 2-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor antagonist YM90K on spatial working memory were investigated by using a delay-interposed radial-arm maze (RAM) task in rats. CGS19755 and MK801, at the largest dose that had no effect on the performance in the nondelayed RAM task, significantly decreased the intial correct response after the 5-min delay in the delay-interposed RAM task (Fig. 2). In contrast, YM90K had no effect on the initial correct response and arm reentries in both the delay-interposed and nondelayed RAM task. CGS19755, MK801 and YM90K, at all doses tested, did not alter the running time in either the delayed or the nondelayed RAM tasks. These results suggest that spatial working memory can be impaired by a blockade of NMDA receprot function and that such impairment is particularly sensitive to delay interposition. The lack of effect of the AMPA receptor antagonist provides additional evidence of the importance of the NMDA subtype of the glutamate receptors in cognitive processes.

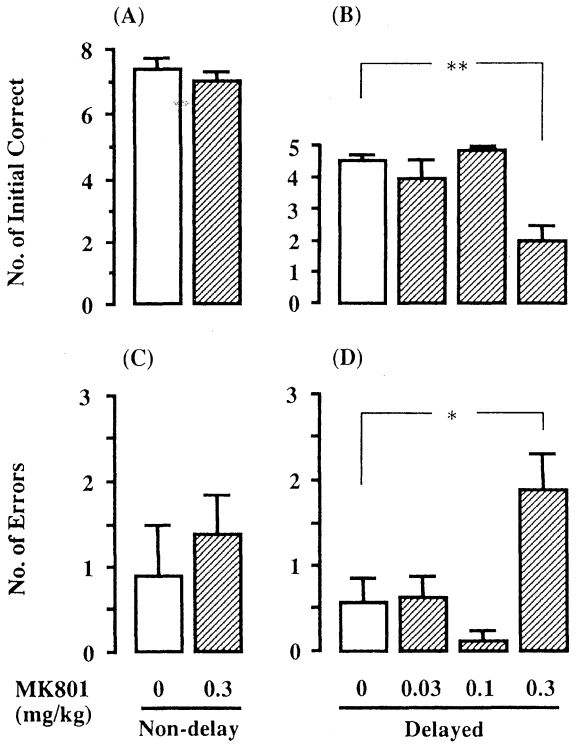

Fig. 2 Effect of MK801 on performance in the delay-interposed and nondelayed RAM tasks by rats. MK801 was administered 15 min before starting the experiments. In the delayed RAM task, the "NO. of Initial Correct" responses were recorded as the number of correct choices before committing the first error. Open bars = control groups; shaded bars = MK801-treated groups. Each value is the mean±SEM of 8-9 rats. $*p<0.05$ and $**p<0.01$ vs. vehicle-treated rats in the delayed-interposed RAM task.

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II．中枢作用薬の神経薬理学的研究

1）長期隔離飼育マウスの攻撃行動発症機序の解明

1-1) Central corticotropin-releasing factor and benzodiazepine receptor systems are involved in the social isolation stress-induced decrease in ethanol sleep in mice:

Social isolation stress has been demonstrated to decrease the hypnotic activity of ethanol in rodents. In this study, the role of central corticotropin-releasing factor (CRF) and $GABA_A$/benezodiazepine (BZD) receptor systems in the social isolation stress-induced decrease in the hypnotic activity of ethanol in mice was investigated by examining the effect of α-helical CRF_{9-41} (αhCRF) and flumazenil, antagonists of CRF and BZD receptors, respectively, on ethanol-induced sleep in group-housed and socially isolated mice. We also tested whether social isolation stress affects the ability of ethanol to enhance the GABA-induced $^{36}Cl^-$ influx into a synaptoneurosomal preparation of mouse forebrain. Social isolation stress significantly decreased both the ethanol (4 g/kg i.p.)-induced and pentobarbital (50 mg/kg i.p.)-induced sleeping times, while this stress had no effect on chloral hydrate (325 mg/kg i.p.)-induced sleep. The i.c.v. injection of αhCRF (6.5 nmol) and flumazenil (33 nmol) antagonized the social isolation stress-induced decrease in the ethanol sleep without affecting ethanol sleep in group-housed animals. Social isolation stress significantly attenuated the ability of GABA to stimulate $^{36}Cl^-$ influx but this stress had no effect on the ability of ethanol to enhance GABA-induced $^{36}Cl^-$ influx. These results suggest that the functional changes in central CRF and $GABA_A$/BZD receptor systems are involved in the social isolation stress-induced decrease in the hypnotic activity of ethanol in mice.

1-2) Flumazenil reverses the decrease in the hypnotic activity of pentobarbital by social isolation stress: are endogenous benzodiazepine receptor ligands involved?:

Long-term social isolation stress has been shown to cause a decrease in pentobarbital (PB)-induced sleeping time in mice. In the present study, to clarify whether the $GABA_A$/benzodiazepine (BZD) receptor system is involved in the decrease in the hypnotic activity of PB by social isolation stress, we examined the effects of BZD receptor ligands on the PB-induced sleep in group-housed and socially isolated mice. Moreover, we also tested whether social isolation stress affects the ability of GABA to stimulate $^{36}Cl^-$ uptake or the modulatory effect of diazepam and PB on GABA-induced stimulation of $^{36}Cl^-$ uptake into synaptoneurosomes prepared from mouse brain. Social isolation stress significantly decreased the PB-induced sleeping time in mice. The BZD receptor agonist diazepam (0.1-0.8 mg/kg, i.p.) dose-dependently prolonged PB sleep in group-housed and isolated mice, but the effect was weaker in isolated mice. In contrast, FG7142 (5-10 mg/kg, i.p.), a BZD receptor inverse agonist, shortened the sleep in group-housed but not in isolated mice. Flumazenil (16.5-33 nmol, i.c.v), a selective BZD receptor antagonist, caused PB sleep in isolated mice to return to the level of group-housed mice, at the dose that antagonized the effects of diazepam and FG7142 on PB sleep in group-housed mice. However, this antagonist alone produced no effect on PB sleep in group-housed mice. Social isolation stress decreased the ability of GABA (0.6-200 μM) to stimulate $^{36}Cl^-$ uptake into synaptoneurosomes but this stress had no effect on PB- and diazepam-induced enhancement of GABA-stimulated $^{36}Cl^-$ uptake. These results suggest that endogenous substance(s) with an inverse BZD receptor agonist-like property and the changes in the ability of GABA to stimulate chloride ion channels are involved in the decrease in the hypnotic activity of pentobarbital following social isolation stress.

1-3) Flumazenil but not FG7142 reverses the decrease in pentobarbital sleep caused by activation of central noradrenergic systems in mice:

Central noradrenergic systems have been shown to modulate the hypnotic activity of pentobarbital in mice. To determine whether the $GABA_A$/banzodiazepine receptor system is involved in the decrease in pentobarbital sleep caused by activation of central noradrenergic systems, we examined in mice

the effects of the benzodiazepine receptor ligands flumazenil and FG7142 on pentobarbital-induced sleep, and on adrenoceptor ligand modulation of pentobarbital sleep. The intracerebroventricular (i.c.v) administration of methoxamine (8-200 nmol), an α_1-adrenoceptor agonist, and yohimbine (1-30 nmol), an α_2-adrenoceptor antagonist, produced a dose-dependent decrease in sleeping time induced by pentobarbital (50 mg/kg, intraperitoneally (i.p.)). The i.c.v. administration of flumazenil (16.5 and 33 nmol), a selective benzodiazepine receptor antagonist, had no effect on pentobarbital sleep, whereas an i.p. injection of FG7142, a selective benzodiazepine receptor inverse agonist, shortened pentobarbital sleep. Flumazenil (33 nmol, i.c.v.) caused the pentobarbital sleep time, shortened by methoxamine (200 nmol, i.c.v.) and yohimbine (30 nmol, i.c.v.), to return to the control level, while FG7142 (10 mg/kg, i.p.) had no effect on the methoxamine- and yohimbine-shortened pentobarbital sleep (Fig. 3). These results suggest that putative endogenous benzodiazepine receptor ligands with an inverse agonist-like property are involved in the methoxamine- and yohimbine-induced decrease in pentobarbital sleep in mice.

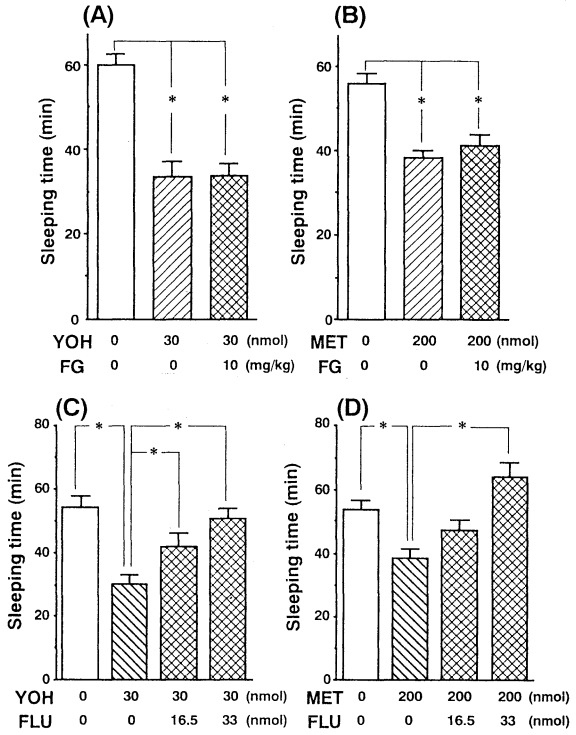

Fig. 3 Effects of FG7142 and flumazenil on yohimbine- and methoxamine-induced decreases in pentobarbital sleep in mice. Either vehicle or FG7142 (FG; 10 mg/kg) was injected i.p. 60 min before pentobarbital. Yohimbine (YOH; 30 nmol, i.c.v.), methoxamine (MET; 200 nmol, i.c.v.) or vehicle was injected i.c.v. 30 min before pentobarbital. Flumazenil (FLU; 16.5 and 33 nmol) or vehicle was co-administered with YOH (C: 30 nmol, i.c.v.), MET (D; 200 nmol, i.c.v.) or vehicle 30 min before pentobarbital injection. Pentobarbital (50 mg/kg) was injected i.p., and the duration of pentobarbital sleep was measured. Each datum represents the mean±S.E.M. (n=9-10). *P<0.05.

2) 中枢セロトニン神経制御機構に関する研究

α2-Adrenoceptor Antagonists Reverse the 5-HT_2 Receptor Antagonist Suppression of Head-Twitch Behavior in Mice:

The α2-adrenoceptor agonist clonidine, as well as 5-HT_2 receptor antagonists, reportedly suppress 5-HT_2 receptor-mediated head-twitch behavior. We investigated the effect of α2-adrenoceptor antagonists on the suppressive action of 5-HT_2 receptor antagonists in mice pretreated with the noradrenaline toxin 6-hydroxydopamine (6-OHDA) or the 5-HT synthesis inhibitor p-chlorophenylalanine (p-CPA). In normal mice, idazoxan (0.08-0.2 mg/kg, IP) or yohimbine (0.2-2.0 mg/kg, IP), both α2-adrenoceptor antagonists, had no effect on the head-twitch response caused by 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT; 16 mg/kg, IP), but idazoxan significantly enhanced the response at 0.5 mg/kg. On the other hand, these α2-adrenoceptor antagonists, at doses that had no effect on the basal number of head-twitches (idazoxan 0.2 mg/kg and yohimbine 0.5 mg/kg), significantly attenuated not only the suppressive effect of clonidine (0.01 mg/kg, IP) on head-twich response but also that of the 5-HT_2 receptor antagonist ritanserin (0.03 mg/kg, IP). Moreover, idazoxan (0.2 mg/kg) also significantly reversed the inhibition by 0.01 mg/kg (IP) ketanserin, a selective 5-HT_2 receptor antagonist. Pretreatment with 6-OHDA plus nomifensine but not with p-CPA significantly attenuated the effect of idazoxan (0.2-0.5 g/kg) on the ritanserin inhibition of the head-twitch response. Prazosin, an α1-adrenoceptor antagonist, dose-dependently suppressed the response, and the effect of prazosin (1.25

mg/kg) was significantly attenuated by 0.5 mg/kg idazoxan. These results indicate that endogenous noradrenaline is involved in the apparent antagonistic interaction between selective α2-adrenoceptor antagonists and 5-HT_2 receptor antaognists in the head-twith response, and suggest that noradrenaline stimulation of α1-adrenoceptors may be involved in this apparent antagonism.

3）線条体アセチルコリン遊離機序の解明

Effect of Pilocarpine on Striatal Acetylcholine Release in Dopamine-Depleted Rats:

We have previously demonstrated that the systemic administration of pilocarpine stimulates striatal acetylcholine (ACh) release in rats using a brain microdialysis technique. In the present study, we investigated whether a nigro-striatal dopaminergic system is involved in the pilocarpine-induced increase in striatal ACh release using dopamine -depleted rats under urethane anesthesia. The application of pilocarpine (0.1-10 mM) *via* the microdialysis tube increased striatal ACh release in normal rats in a concentration-dependent manner, but it had no effect on the release of glutamate or γ-aminobutyric acid (GABA) from the striatum. The increase in striatal ACh release caused by pilocarpine (1 mM) was enhanced by reserpine and α-methyl-*p*-tyrosine treatment, which completely depleted dopamine in the striatum. These results suggest that pilocarpine selectively increases striatal ACh release by acting at the striatum, and that the nigro-striatal dopaminergic neurons play an inhibitory role in the pilocarpine-induced ACh release.

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Ⅲ. ベトナム人参の主要成分 majonoside-R2 の作用

1）隔離飼育ストレス誘発反応に対する majonoside-R2 の作用

Majonoside-R2 (MR2) is a major ocothillol-type seponin constituent of Vietmamese ginseng. We investigated the effect of MR2 on the social isolation stress-induced decrease in pentobarbital sleep in mice, and elucidated the possible involvement of neurosteroidal sites of the $GABA_A$ receptor complex in the pharmacological activity of MR2. MR2 (3.1-6.2 mg/kg, i.p. or 5-10 μg, i.c.v.) dose-dependently reversed the decrease in pentobarbital sleep caused by social isolation stress to the level of sleep in the group-housed mice, but it had no effect on pentobarbital sleep in group-housed mice. Allotetrahydrodeoxycorticosterone (5α-pregnane-3α, 21-diol-20-one, allo-THDOC; 12.5 μg, i.c.v.), the positive allosteric modulator of the $GABA_A$ receptor, and α-helical CRF_{9-41} (αhCRF; 25 μg,i.c.v.), the corticotropin-releasing factor (CRF) antagonist, also reversed the decrease in pentobarbital sleep caused by social isolation stress. The reversing effects of i.c.v. MR2 and i.c.v. allo-THDOC on the decrease in pentobarbital sleep in isolated mice were significantly attenuated by pregnenolone sulfate (10 μg, i.c.v.), the steroidal negative allosteric modulator of the $GABA_A$ receptor (Fig. 4). In contrast, when injected i.c.v., MR2, as well as allo-THDOC and αhCRF, significantly reversed the decrease in pentobarbital sleep induced by pregnenolone sulfate (10 μg,i.c.v.) and CRF (10 μg, i.c.v.) in group-housed mice. These results suggest that the reversing effect of MR2 on the social isolation stress-induced decrease in pentobarbital sleep is mediated by the neurosteroid site on the $GABA_A$ receptor complex in mice.

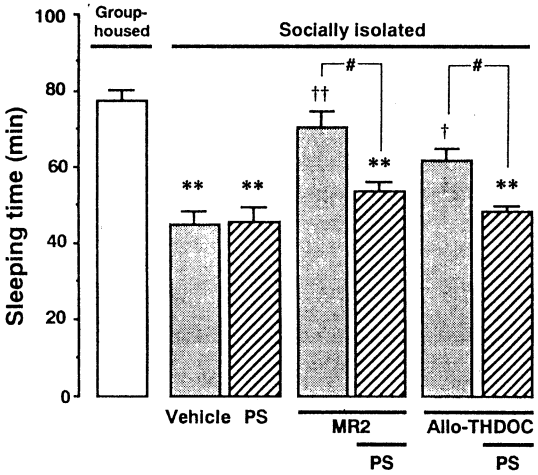

Fig. 4 Antagonistic interaction between pregnenolone sulfate and majonoside-R2 or allo-THDOC in pentobarbital sleep in isolated mice. Animals were isolated for 5-7 weeks before the experiments. Pregnenolone sulfate (PS, 10 μg) was coadministered i.c.v. with majonoside-R2 (MR2, 10 μg) or allo-THDOC (12.5 μg) 30 min before pentobarbital (50 mg/kg, i.p.). Each datum represents the mean±SEM of 8-10 mice. **$p<0.01$ compared with group-housed mice. †$p<0.05$ and ††$p<0.01$ compared with vehicle-treated group. #$p<0.05$ compared with respective MR2 or allo-THDOC alone (three-way ANOVA followed by Tukey's test).

2）クロニジンの抗侵害受容作用に対する majonoside-R2 の作用

Majonoside-R2 (MR2) is a major constituent of Vietnamese ginseng (*Panax vietnamensis,* Ha et Grushv. Araliaceae) that is known to exhibit antagonistic activity against opioid-induced antinociception. In this study, we investigated the effect of MR2 on the antinociception caused in mice by the α2-adrenoceptor agonist clonidine, and elucidated the role of supraspinal GABAergic systems in this effect of MR2. The systemic administration of clonidine (0.5-2.5 mg/kg, s.c.) dose-dependently suppressed the nociceptive response of mice in the tail-pinch and hot-plate tests. The intraperitoneal (i.p.), intracerebroventricular (i.c.v.) or intrathecal (i.t.) administration of idazoxan (a selective α2-adrenoceptor antagonist) significantly antagonized the antinociceptive effect of clonidine in both tests. MR2 administered systemically (1.5-6.2 mg/kg, i.p.) or centrally (5-10 μg/mouse, i.c.v. or i.t) dose-dependently antagonized the clonidine (1 mg/kg, s.c.)-induced antinociception in the tail-pinch test but not in the hot-plate test. The antagonistic effect of i.c.v. MR2 on the systemic clonidine-induced antinociception in the tail-pinch test was significantly reversed by i.c.v. administrations of the selective benzodiazepine receptor antagonist flumazenil (5 μg/mouse) and the $GABA_A$ antagonist picrotoxin (0.25 μg/mouse) (Fig. 5). These results suggest that the supraspinal $GABA_A$/benzodiazepine receptors are

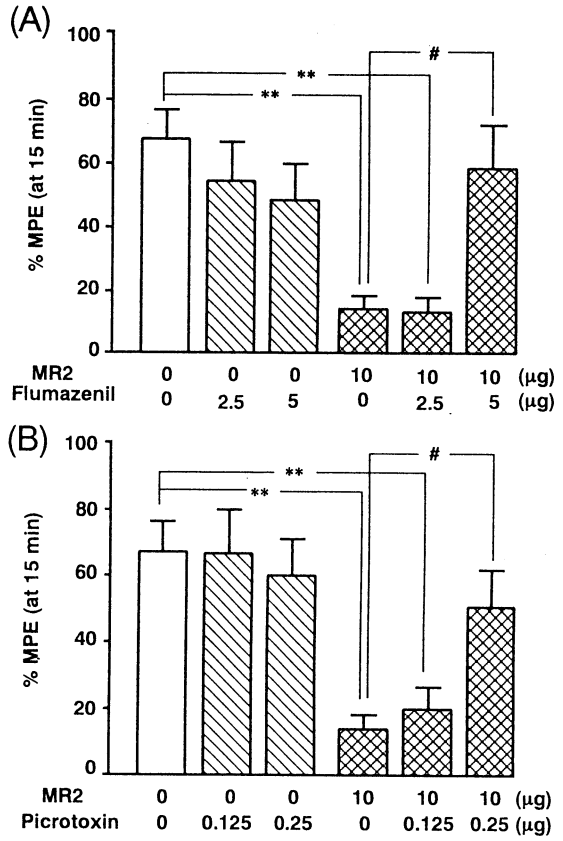

Fig. 5 Reversal by flumazenil and picrotoxin of the suppressive effect of majonoside-R2 (MR2) on the clonidine-induced antinociception in the tail-pinch test at the supraspinal level. After the basal nociceptive responses in the tail-pinch test were recorded, clonidine (1 mg/kg, s.c.) was administered. The letency of the nociceptive responses was measured at 15 min after clonidine administration. MR2 (10μg/mouse) was co-administered i.c.v. with or without 2.5-5 μg/mouse flumazenil (A) and 0.125-0.25 μg/mouse picrotoxin (B) just before clonidine. Each column represents the mean %MPE ± S.E.M. (n=10). **P<0.01 vs. vehicle groups. #P<0.05 vs. MR2 alone (Tukey's test).

involved in the antagonistic effect of MR2 on the clonidine-induced antinociception in the tail-pinch test in mice.

3) ベトナム人参の貧食作用

The effects of Vietnamese ginseng crude extract (VG extract), total saponin (VG saponin) and its major saponin component, majonoside-R2, on phagocytosis were examined in mice by bactericidal and carbon clearance tests. *Escherichia coli* (*E. coli*) ATCC 25922 was used to induce the acute toxicity and activate the phagocytic activity of phagocytes in both *in vitro* and *in vivo* bactericidal tests. Pretreatment with VG extract (500 mg/kg, oral administration, *p.o.*) and majono-side-R2 (50 mg/kg, intraperitoneal administration, *i.p.*) protected the animals from the acute toxicity of *E. coli* ATCC 25922 and significantly increased the phagocytic index in both *in vitro* and *in vivo* bactericidal tests (Fig. 6). Moreover, VG extract (100-500 mg/kg,p.o.), VG saponin (25 mg/kg, *i.p.*) and majonoside-R2 (10 mg/kg, *i.p.*), as well as zymosan A, a non-specific phagocytic stimulant, also increased

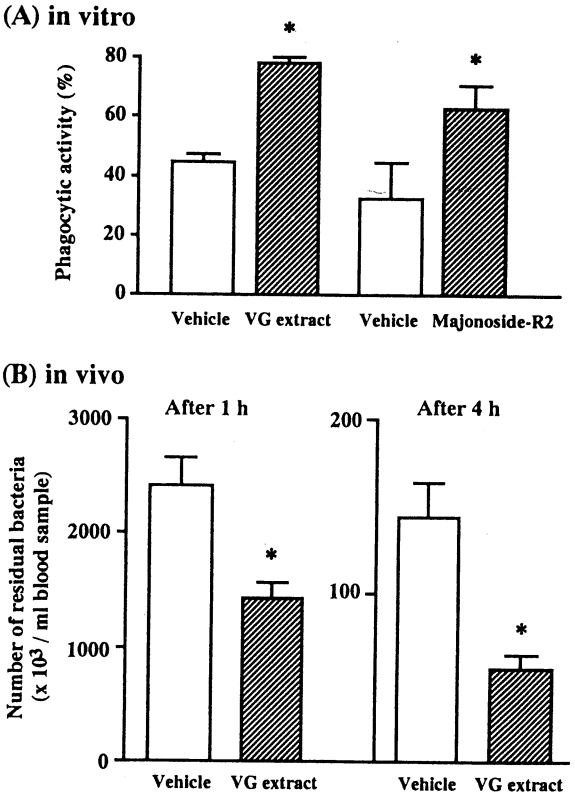

Fig. 6 Effect of Vietnamese ginseng extract (VG extract) and majonoside-R2 on phagocytosis in both *in vitro* (A) and *in vivo* (B) bactericidal tests. Vehicle, VG extract (500 mg/kg, *p.o.*) or majonoside-R2 (50 mg/kg,*i.p.*) was administered twice a day for 5 days (11 times in total) before assays. *Escherichia coli* ATCC 25922 was applied to activate phagocytosis in the concentrations of 3×10^8 and 5×10^7 in *in vitro* or *in vivo* test, tespectively. Blood samples were taken 1 h(A) or 1 h and 4 h(B) after the last administration of test drugs. The phagocytic activity was expressed as the percentage of leukocytes containing bacteria per 100 leukocytes (% phagocytic activity) in (A) or the number of residual bacterial colonies (bacteria/ml blood sample) in (B). Each column represents the mean± SEM (n=15). *P<0.05 vs. vehicle groups (Student's *t*-test).

the phagocytic index evaluated by the carbon clearance test. These results indicate that Vietnamese ginseng enhances the phagocytic activity of phagocytes, and suggest that majonoside-R2 plays an important role in this effect.

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Ⅳ．各種薬用植物の薬理作用の研究

1）タイ薬用植物 Hunteria zeylanica 葉の主成分 corymine 及びその類似化合物の glycine 電流に対する作用

We previously reported that corymine, an alkaloidal compound extracted from the leaves of *Hunteria zeylanica* native to Thailand. potentiated convulsions induced by either picrotoxin or strychnine. Therefore, to clarify the mechanism of action of cyrymine, the effects of corymine on γ-aminobutyric acid (GABA) and glycine receptors were examined. We used *Xenopus* oocytes expressing these receptors and the two-electrode voltage-clamp method. The receptors expressed in oocytes injected with rat brain and spinal cord RNA showed the pharmacological properties of $GABA_A$ and glycine receptors, respectively. Corymine (1-100

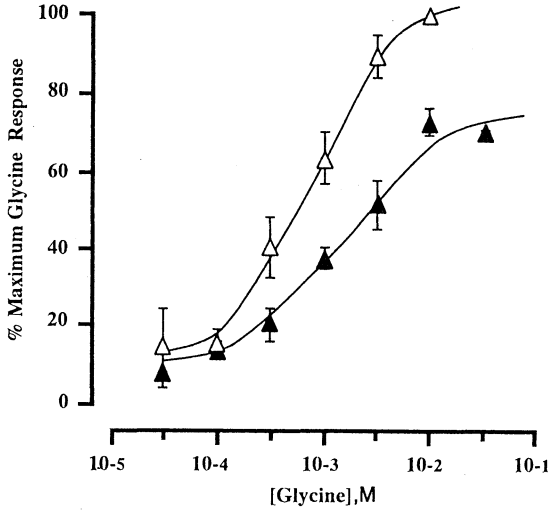

Fig. 7 Antagonism by corymine of glycine concentration-response curve in *Xenopus* oocytes injected with rat spinal cord RNA. Oocytes were treated with glycine (30 μM-30 mM) in the absence (△) or presence (▲) of 30 μM corymine. Data are expressed as percentages of the response elicited by 10 mM glycine as a control. Each point represents data from one oocyte and the mean±S.E. for 2-5 oocytes.

μM) partially (20-30 %) reduced the GABA responses in oocytes injected with rat brain RNA. while marked (up to 80 %) dose-dependent reductions were observed in the glycine responses in oocytes injected with rat spinal cord RNA. These observations suggest that corymine was more effective against the glycine receptors than the GABA receptors. The ED_{50} of corymine on the glycine response was 10.8 μM. Corymine, at 30 μM, caused a shift to the right, with a lower maximal response, of the glycine concentration-response curve (Fig. 7). This indicated that the action of corymine on glycine receptors is neither competitive nor purely non-competitive. These observations suggest that a binding site other than the glycine recognition site of the glycine receptors is the site of action of corymine.

2) タイ薬用植物 Mitragyna speciosa 葉の主要アルカロイド成分 mitragynine の抗侵害受容

Mitragynine (MG), a major alkaloidal constituent extracted from the plant *Mitragyna speciosa* Korth, is known to exert an opioid-like activity. Our previous study showed the involvement of opioid systems in the antinociceptive activity of MG in the tail-pinch and hot-plate tests in mice. In the present study, to clarify the opioid receptor subtypes involved in the antinociceptive action of MG, we investigated the effects of selective antagonists for μ-, δ-and κ-opioid receptors on antinociception caused by the intracerebroventricular (i.c.v.) injection of MG in the tail-pinch and hot-plate tests in mice. The coadministration of a selective μ-opioid antagonist, cyprodime (1-10 μg, i.c.v.) and the pretreatment with a selective μ1-opipod antagonist naloxonazine (1-3 μg, i.c.v.) significantly antagonized the antinociceptive activities of MG (10 μg, i.c.v.) and morphine (MOR, 3 μg, i.c.v.) in the tail-pinch and hot-plate tests. Naltrindole (1-5 ng. i.c.v.), a selective δ-opioid antagonist, also blocked the effects of MG (10 μg, i.c.v.) without affecting MOR (3 μg, i.c.v.) antinociception (Fig. 8). Nor-binaltorphimine, a selective κ-opioid antagonist, significantly attenuated MG (10 μg, i.c.v.) antinociception in the tail-pinch test but not in the hot-plate test at the dose (1 μg, i.c.v.) that antagonized the antinociceptive effects of the selective κ-opioid agonist U50,488H in both tests, while it had no effect on MOR antinociception in either tests. These results suggest that antinociception caused by i.c.v. MG is dominantly mediated by μ- and δ-opioid receptor subtypes, and that the selectivity of MG for the supraspinal opioid receptor subtypes differs from that of MOR in mice.

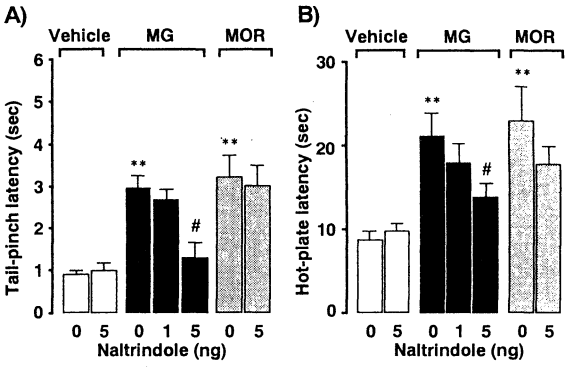

Fig. 8 Effects of the δ-opioid antagonist naltrindole on i.c.v.-administered mitragynine- and morphine -induced antinociception in the tail-pinch (A) and hot-plate (B) tests in mice. Mitragynine (MG : 10 μg, i.c.v.) or morphine (MOR : 3 μg, i.c.v.) or vehicle was coadministered with naltrindole (1-5 ng). After 15 min, the latency of the nociceptive responses was measured. Each data column represents the mean±S.E.M. of 7-9 mice. **P<0.01 compared with the animals administered vehicle alone. #P<0.05 compared with MG or MOR alone (Mann-Whitney U-test).

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