生 物 試 験 部 門

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◇研究目的

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基礎研究，和漢薬の中枢作用を定量的に評価し，その作用本体を追求する研究及びその作用機序を分子レベルで明らかにすることを目的とした研究を行っている。

◇研究概要

Ⅰ．和漢薬の新しい薬効評価法を確立するための基礎的研究

1）脳血管性痴呆病態モデル動物の確立とそれによる和漢薬および抗痴呆薬の薬効評価

2）情動ストレス反応の不安・うつ病態モデルとしての応用と和漢薬作用

3）新規リード化合物の開発をめざした各種民族薬の薬理作用の探索と作用機構の解析

Ⅱ．中枢作用薬の神経薬理学的研究

1）心理的ストレス反応に関わる神経機構，受容体機能修飾因子，分子機序の解析

2）神経伝達物質の脳内動態および代謝回転の解析と薬物作用

Ⅲ．遺伝子発現を指標とした薬物作用の解明と和漢薬作用に関する研究

1）慢性脳虚血により発現する脳内遺伝子のクローニングとその機能解析

2）うつ病態関連脳内遺伝子の発現変化と抗うつ薬・和漢薬の作用解析

◇著　書

1) Watanabe H., Matsumoto K. and Matsuda H.: Pharmacological study of the anti-dementia effect of Shimotsu-to (Si-Wu-Tang). In Pharmacological Research on Traditional Herbal Medicines, by Watanabe H. and Shibuya T. (Eds) 55-65, harwood academic publishers, 1999.
2) Nguyen T. T. H., Nguyen T. N., Matsumoto K., Yamasaki K. and Watanabe H.: Effects of Vietnamese ginseng on psychological stress-induced changes in pharmacological responses. In Pharmacological Research on Traditional Herbal Medicines, by Watanabe H. and Shibuya T. (Ebs) 77-92, harwood academic publishers, 1999.

◇原　著

1) **Li H.-B., Matsumoto K. and Watanabe H.: Different effects of unilateral and bilateral hippocampal lesions in rats on the performance of radial maze and odor-paired associate tasks. Brain Res. Bull. 48 : 113–119, 1999.**

Abstract : The hippocampus plays an important role in the declarative or explicit memory in humans and is necessary for allocentric spatial learning and olfactory memory in animals. In primates and rodents, the bilateral hemispheres of the brain (especially the forebrain) symmetrically and asymmetrically contribute to diverse cognitive manipulations. In this study, we investigated the role of the hippocampus in spatial memory and in odor-paired associate memory by unilaterally or bilaterally lesioning this region in rats. The bilateral removal, but not the unilateral removal, of the hippocampus impaired both the acquisition of spatial working memory in the radial maze task and the retrieval of maze performance tested 1 month after the acquisition trials. In contrast, neither bilateral nor unilateral removal impaired the odor-paired associate learning. These findings suggest that the hippocampus is critical to the spatial memory, and that a unilateral hippocampus is sufficient for executing a spatial task. The present results also indicate that the hippocampus plays a minor role in odor-dominated associate learning and that some kinds of memories in rats may be processed independently by the left or right hippocampus.

2) **Dong E., Matsumoto K., Tohda M., Kaneko Y. and Watanabe H.: Diazepam binding inhibitor (DBI) gene expression in the brains of socially isolated and group-housed mice. Neurosci. Res. 33 : 171–177, 1999.**

Abstract : Diazepam binding inhibitor (DBI), a putative endogenous polypeptide ligand for benzodiazepine (BZD) receptors, has been shown to act as an inverse BZD receptor agonist in the brain. We previously suggested that the social isolation stress-induced decrease in pentobarbital sleeping time in mice was partly due to an increase in the activity of endogenous substances with an inverse BZD receptor agonist-like property such as DBI. In this study, we examined whether the DBI gene expression is affected by socially isolated stress. Consistent with the previous findings, the in situ hybridization result showed very strong signals of DBI mRNA around the regions of the third ventricle, especially the lining cells, the arcuate

nucleus of the hypothalamus and the cerebellum, in both socially isolated and group-housed animals. Unexpectedly, however, semi-quantitative experiments with reverse transcription polymerase chain reaction technique revealed that socially isolated mice had significantly less expression of DBI mRNA in the hypothalamus than group-housed animals, and no difference in the expression in the other brain areas was observed between two animal groups. We discuss the relationship between the decrease of DBI mRNA expression in the hypothalamus and the decrease of $GABA_A$ receptor function following long-term social isolation in mice.

3) **Dong E., Matsumoto K., Tohda M. and Watanabe H.: Involvement of diazepam binding inhibitor and its fragment octadecaneuropeptide in social isolation stress-induced decrease in pentobarbital sleep in mice. Life Sci. 64 : 1779–1784, 1999.**

Abstract : Diazepam binding inhibitor (DBI) and its fragment, octadecaneuropeptide (ODN), are putative endogenous ligands for benzodiazepine (BZD) receptors and have been shown to act as an inverse BZD receptor agonist in the brain. A previous study suggested that the social isolation stress-induced decrease in pentobarbital sleep in mice was partly due to endogenous substances with an inverse BZD receptor agonist-like property. In this study, we examined the effects of DBI and ODN on pentobarbital sleep in group-housed and socially isolated mice to test the possible involvement of DBI and ODN in a social isolation-induced decrease in pentobarbital sleep. The socially isolated mice showed significantly shorter durations of pentobarbital (50 mg/kg, intraperitoneally, i. p.) sleep compared to the group-housed animals. When injected intracerebroventricularly (i.c.v.), DBI and ODN (3 and 10 nmol) dose-dependently shortened the pentobarbital-induced sleeping time in group-housed mice at the same dose range, but these peptides had no effect on the sleeping time in socially isolated animals. In contrast, flumazenil (16.5-33 nmol, i.c.v.), a BZD receptor antagonist, reversed the pentobarbital sleeping time in socially isolated mice to the level of group-housed animals without affecting the sleeping time in group-housed animals. The effects of DBI and ODN in group-housed mice were significantly blocked by flumazenil (33 nmol, i.c.v.). Moreover, the effect of flumazenil in socially isolated mice was significantly attenuated by DBI and ODN (10 nmol, i.c.v.). These results suggest that the changes in the activity of DBI and/or ODN are partly involved in the social isolation-induced decrease in the hypnotic action of pentobarbital in mice.

4) **Tohda M., Zhao Q. and Watanabe H.: Influence of chronic treatment with imipramine on mRNA levels in rat brain: elevation of glyceraldehyde-3-phosphate dehydrogenase levels. Jpn. J. Pharmacol. 81 : 393–396, 1999.**

Abstract : The differential display method was used to identify the intrinsic factor that changes its mRNA expression level in rat brain after a 14-day oral administration of 20 mg/kg imipramine. The expression of a 180-bp band was markedly enhanced by imipramine. The results of sequencing and a data base search revealed that the isolated clone was

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with a one-base difference. Enhancement of the expression by imipramine was observed in the amygdala. Quantitative PCR showed that imipramine treatment significantly elevated the GAPDH/β-actin ratio in the cortex. These findings suggest that long-term treatment with imipramine stimulates GAPDH mRNA expression.

5) **Dong E., Matsumoto K. and Watanabe H.: Involvement of peripheral type of benzodiazepine receptor in social isolation stress-induced decrease in pentobarbital sleep in mice. Life Sci. 65 : 1561–1568, 1999.**

Abstract : Our previous studies have shown that central-type benzodiazepine (BZD) receptors (CBR) and neurosteroids capable of modulating $GABA_A$ receptor function are involved in the decrease of pentobarbital (PB)-induced sleep caused by social isolation stress in mice. In this study, to further clarify the mechanism underlying this decrease, we investigated the possible involvement of peripheral-type BZD receptors (PBR) which play an important role in neurosteroidogenesis in PB sleep in socially isolated mice. Socially isolated mice showed significantly shorter duration of PB-induced sleep than group-housed animals. When injected intracerebroventricularly (i.c.v.), FGIN-1-27 (FGIN, 25-100 nmol), a selective PBR agonist, and PK11195 (PK, 14-28 nmol), a PBR antagonist, and pregnenolone (PREG, 15-30 nmol), a neurosteroid precursor, dose-dependently normalized the PB sleep in isolated mice without having an effect on the group-housed animals. In contrast, pregnenolone sulfate (PS, 24 nmol), an endogenous neurosteroidal negative allosteric modulator of the $GABA_A$ receptor, reduced PB sleep in group-housed but not isolated mice. PS, at the same dose, significantly attenuated the effects of FGIN (100 nmol), PK (28 nmol) and PREG (30 nmol) in isolated mice, while FGIN (100 nmol), PK (28 nmol) and pregnenolone (30 nmol) significantly blocked the effect of PS (24 nmol) in group-housed mice. These results suggest that the PBR-mediated decrease in the genesis of neurosteroid(s) possessing a $GABA_A$ receptor agonistic profile is also partly involved in the down regulation of the $GABA_A$ receptor following long-term social isolation and contributes to the decrease of PB-induced sleep in isolation stressed mice.

6) **Matsumoto K., Yobimoto K., Nguyen T.T.H., Abdel-Fattah Mohamed A.-F., Tran V. H. and Watanabe H.: Psychological stress-induced enhancement of brain lipid peroxidation via nitric oxide systems and its modulation by anxiolytic and anxiogenic drugs in mice. Brain Res. 839 : 74–84, 1999.**

Abstract : We investigated the effect of psychological stress on lipid peroxidation activity in the mouse brain, the mechanism underlying the psychological stress-induced change in the activity, and the effects of anxiolytic and anxiogenic drugs on the activity in psychologically-stressed animals. Psychological stress exposure using a communication box paradigm for 2-16 h significantly increased the content of thiobarbituric acid reactive substance (TBARS), an index of lipid peroxidation activity, in the brain, and the effect was maximal

after peaked by a 4-h stress exposure. In the animals stressed for over 4 h, the increased brain TBARS content lasted for 30 min after the stress exposure, while no significant increase of the TBARS content was observed in the liver or serum. Trolox (67.6 mg/kg, i.p.), an antioxidant drug, but not monoamine oxidase inhibitors, clorgyline (2.5-5 mg/kg, i.p.) or 5-(4-benzylphenyl)-3-(2-cyanoethyl)-(3*H*)-1,3,4-oxadiazol-2-one (1-5 mg/kg, i.p.), significantly suppressed the effect of psychological stress. The non-selective nitric oxide (NO) synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 10-100 mg/kg, i.p.) and the selective neuronal NOS inhibitor 7-nitroindazole (25 and 50 mg/kg, i,p.), but not the inducible NOS inhibitor aminoguanidine (1-100 mg/kg, i.p.), dose dependently suppressed the psychological stress-induced enhancement of lipid peroxidation in the brain. L-Arginine (300 mg/kg, i.p.), a substrate of NOS, antagonized the effect of L-NAME. Measurements of NO metabolites revealed a significant increase of NO production in the brains of stressed mice. The benzodiazepine (BZD) receptor agonist diazepam (0.05-0.5 mg/kg, i.p.), the 5-HT_{1A} receptor agonists (±)-8-hydroxy-di-propylaminotetralin and buspirone (0.1-1 mg/kg, i.p.), but not the 5-HT_3 receptor agonist MDL72222, dose-dependently suppressed the psychological stress-induced enhancement of brain lipid peroxidation. In contrast, the administration of anxiogenic drugs, FG7142 (an inverse BZD agonist: 1-l0 mg/kg, i.p.) and l-(3-chlorophenyl)piperazine (a mixed 5-$HT_{2A/2B/2c}$ agonist: 0.1-1 mg/kg, i.p.), potentiated it. The effects of diazepam and FG7142 were abolished by the BZD receptor antagonist flumazenil (10 mg/kg, i.p.). These results indicate that psychological stress causes oxidative damage to the brain lipid via enhancing constitutive NOS-mediated production of NO, and that drugs with a BZD or 5-HT_{1A} receptor agonist profile have a protective effect on oxidative brain membrane damage induced by psychological stress.

7) **Matsumoto K., Uzunova V, Pinna G, Taki K., Uzunov D.P., Watanabe H., Mienville J.M., Guidotti A. and Costa E.: Permissive role of brain allopregnanolone content in the regulation of pentobarbital-induced righting reflex loss. Neuropharmacol. 38 : 955–963, 1999.**

Abstract: Allopregnanolone [3 α -hydroxy-5 α -pregnan-20-one] (ALLO), a potent neurosteroid that positively modulates γ -aminobutyric acid (GABA) action at various $GABA_A$ receptor subtypes is synthesized in nanomolar concentrations and stored non uniformly in various brain structures of mammals. We have measured brain ALLO content and its precursors by negative ion chemical ionization-mass-spectrometry after purification and separation of the different steroids with HPLC and gas chromatography. Our procedure measures steroids in the femtomolar range with structural information and unsurpassed selectivity. We were able to establish an association between the decrease in content of ALLO in mouse brain cortex elicited by either long-lasting social isolation or by the administration of 17 β -17[bis (1-methylethyl) amino carbonyl] androstane-3,5-dilene-3-carboxylic acid (SKF105111), an inhibitor of Types I and II 5 α reductases, and the shortening of the righting reflex loss elicited by pentobarbital (PBT). SKF105111 added to cortical brain slices in

concentrations up to 10^{-5} M failed per se to alter GABAergic currents or their potentiation by PTB recorded from pyramidal neurons. Fluoxetine (1.45 or 2.9 μmol/kg i.p.) doses that fail to change the PTB-induced loss of righting reflex and the level of brain ALLO in group-housed mice normalized both parameters in socially-isolated mice. In addition, we could detect both fluoxetine actions in socially isolated mice pretreated with doses of *p*-chlorophenylalanine (1.2 mmol/kg i.p. at 72, 48, and 24 h) that substantially inhibit brain serotonin 5HT synthesis as shown by an 80% drop of brain 5HT content. These studies for the first time have provided evidence suggesting that the endogenous cortical stores of ALLO physiologically upregulate GABAergic tone and by such a mechanism play a permissive or facilitatory role on the PTB-induced loss of the righting reflex. In the absence of such a permissive physiological influence by endogenous ALLO, the righting reflex inhibition by PTB is down regulated.

◇総　説

1）南里真人, 渡辺裕司: 慢性脳循環障害モデルとしての両側総頸動脈永久結紮ラットの有用性. 日本薬理学雑誌, 113： 85–95, 1999.

◇学会報告

1）渡辺裕司, 董而博, 松本欣三：Diazepam binding inhibitor (DBI) のマウス血漿中テストステロン及びエストラジオール量に対する抑制効果. 第72回日本薬理学会年会, 1999, 3/22–25, 札幌.

2）李紅彬, 松本欣三, 渡辺裕司, 加藤総夫：ラット海馬 CA1 錐体細胞における NMDA 誘発膜電位変化 －赤外線ビデオ顕微鏡下パッチクランプを用いた検討. 第72回日本薬理学会年会, 1999, 3/22–25, 札幌.

3）金子喜彦, 松本欣三, グエンチー・スー・フォン, 東田道久, プーイアジュリア, 笠井良次, 山崎和男, 渡辺裕司：アフリカツメガエル卵母細胞上に再構築させた $GABA_A$ 受容体に対するベトナム薬用人参の主要成分 Majonoside-R2 の作用. 第72回日本薬理学会年会, 1999, 3/22–25, 札幌.

4）アブデルファッター・アリ, 松本欣三, 村上孝寿, モスタファファイェズ, ハテムガンマーズ, 渡辺裕司：中枢セロトニンの放出, 取り込み及び代謝回転に及ぼす harmine 及び harmaline の影響. 第72回日本薬理学会年会, 1999, 3/22–25, 札幌.

5）松本欣三, 李紅彬, 渡辺裕司：ラット空間認知及び連想記憶に及ぼす単側及両側海馬破壊の影響. 第72回日本薬理学会年会, 1999, 3/22–25, 札幌.

6）東田道久, 渡辺裕司：イミプラミン慢性投与によるラット脳カルモジュリン相同性因子の発現増大作用. 第72回日本薬理学会年会, 1999, 3/22–25, 札幌.

7）呼元香保里, 松本欣三, Nguyen Thi Thu Huong, 渡辺裕司, 笠井良次, 山崎和男: 心理的ストレス負荷マウスの脳内脂質過酸化反応に対するベトナム薬用人参サポニン及び主要成分 majonoside-R2 の効果.日本薬学会第118年会, 1999, 3/29–31, 徳島.

8）Matsumoto K. and Watanabe H.: Psychological stress-induced decrease in pentobarbital sleep and the effect of natural medicines. 国際東洋医学会, 1999, 5/27–30, 東京.

9）田畑恵市，松本欣三，渡辺裕司：抗コリン薬 scopolamine によるラット海馬長期増強現象の抑制とそれに対する芍薬成分 paeoniflorin の効果．第16回和漢医薬学会大会，1999，8/28–29，千葉．

10）Abdel-Fattah Mohamed A.-F.，渡辺裕司，松本欣三：Nigella sativa seed（黒種草子）油の抗侵害受容作用．第16回和漢医薬学会大会，1999，8/28–29，千葉．

11）渡辺裕司，張夢暉，松本欣三：四物湯の学習障害改善効果－マウス受動的回避学習課題による検討－．第16回和漢医薬学会大会，1999，8/28–29，千葉．

12）東田道久：Differential Display 法における単離配列解析の改良．第42回日本神経化学会（広島）大会，1999，9/15–17，広島．

13）Abdel-Fattah Mohamed A.-F.，Matsumoto K.，Takayama H.，Aimi N.，Watanabe H: Uncaria alkaloids improve memory deficit induced by cholinergic dysfunctions in mice．第50回日本薬理学会北部会，1999，10/15–16，仙台．

14）東田道久，渡辺裕司: 慢性脳虚血により発現増大する新規因子 vof-16のクローニングとその脳内分布，機能の検討．第50回日本薬理学会北部会，1999，10/15–16，仙台．

◇その他

1）松本欣三: 月経前症候群に $GABA_A$ 受容体の発現変化が関与するか？．ファルマシア，35：262–263，1999．

◇共同研究

相見則郎、高山廣光：千葉大学薬学部，「タイ薬用植物中のインドールアルカロイド類に関する創薬基礎科学研究」1994，4–

山崎和男，笠井良次：広島大学医学部，グエン・チー・スー・フォン：ベトナム薬用人参センター，「ベトナム人参の薬理作用の研究」1994，4–

Erminio Costa，Alessandro Guidotti：イリノイ州立大学シカゴ校精神医学研究所，「ストレス病態における神経活性ステロイドの役割」1997，4–

◇研究費取得状況

文部省科学研究費，萌芽的研究（代表：渡辺裕司）「慢性脳虚血ラットの記憶障害に関与する内因性物質の分子生物学的研究」50万

特別研究員奨励費（代表：渡辺裕司）「エジプト薬用植物成分の中枢セロトニン神経賦活作用」120万

文部省科学研究費，基盤研究Ｂ（２）（代表：渡辺裕司）「白質脳症モデル動物に関する薬理学的研究」770万

文部省科学研究費，基盤研究Ｃ（２）（代表：松本欣三）「ニューロステロイド系を介した GABA-A 受容体機能制御と新規中枢作用薬への応用」70万

文部省科学研究費，奨励研究（A）（代表：東田道久）「内因性の学習障害誘発因子の単離同定とその生理機能・発現制御機構の解明」90万

平成11年度創造開発研究費（代表：松本欣三）「ストレス病態に対する天然薬物作用の研究」38.9万

◇研究室在籍者

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◇学位取得者

修士：
金子喜彦：アフリカツメガエル卵母細胞上に再構成させた $GABA_A$ 受容体に対するベトナム人参主要サポニン成分 Majonoside-R2 の作用
瀧一洋：隔離飼育ストレスによる中枢 GABA 系機能低下と脳内神経ステロイドの役割
張紹輝：ラット学習行動に対する NS-105（(+)-5-oxo-D-prolinepiperidinamide monohydrate）の作用
松田嘉弘：両側総頸動脈永久結紮ラットの学習行動障害および情動に関する薬理学的研究
呼元香保里：心理的ストレス負荷マウスの脳内脂質過酸化反応及びそれに対する薬物作用
課程博士：
董而博：隔離飼育ストレス誘発のペントバルビタール睡眠短縮における diazepam binding inhibitor（DBI）の役割
李紅彬：海馬およびイオン型グルタミン酸受容体の空間・非空間記憶における役割
論文博士
南里真人：ニコチン性アセチルコリン受容体アゴニスト GTS-21[3-(2,4-dimethoxybenzylidene)-anabaseine]の脳保護作用に関する研究