生物試験部門 Department of Pharmacology

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◇研究目的 Aim of the research projects

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 Research projects

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

◇原　著　Original papers

1）**Dong E., Matsumoto K., Uzunova V., Sugaya I., Takahata H., Nomura H., Watanabe H., Costa E., and Guidotti A.: Brain 5α-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation.** ***Proceedings of the National Academy of Sciences of the United States of America.*** **98: 2849-2854, 2001.**

Abstract : Allopregnanolone (ALLO), is a brain endogenous neurosteroid that binds with high affinity to γ-aminobutyric acid type A ($GABA_A$) receptors and positively modulates the action of GABA at these receptors. Unlike ALLO, 5α-dihydroprogesterone (5α-DHP) binds with high affinity to intracellular progesterone receptors that regulate DNA transcription. To investigate the physiological roles of ALLO and 5α-DHP synthesized in brain, we have adopted a mouse model involving protracted social isolation. In the frontal cortex of mice, socially isolated for 6 weeks, both neurosteroids were decreased by approximately 50%. After administration of (17β)-17-(bis-1-methyl amino carbonyl) androstane-3,5-diene-3-carboxylic acid (SKF105,111), an inhibitor of the enzyme (5α-reductase Type I and II) that converts progesterone into 5α-DHP, the ALLO and 5α-DHP content of frontal cortex of both group-housed and socially isolated mice decreased exponentially to 10%-20% of control values in about 30 min. The fractional rate constants (k h^{-1}) of ALLO and 5α-DHP decline multiplied by the ALLO and 5α-DHP concentrations at any given steady-state estimate the rate of synthesis required to maintain that steady state. After 6 weeks of social isolation, ALLO and 5α-DHP biosynthesis rates were decreased to 30% of the values calculated in group-housed mice. Moreover, in socially isolated mice, the expression of 5α-reductase Type I mRNA and protein was approximately 50% lower than in group-housed mice whereas 3α-hydroxysteroid oxidoreductase mRNA expression was equal in the two groups. Protracted social isolation in mice may provide a model to investigate whether 5α-DHP by a genomic action, and ALLO by a nongenomic mechanism down-regulate the action of drugs acting as agonists, partial agonists, or positive allosteric modulators of the benzodiazepine recognition sites expressed by $GABA_A$ receptors.

2）**Tabata K., Matsumoto K., Murakami Y. and Watanabe H.: Ameliorative effects of paeoniflorin, a major constituent of peony root, on adenosine A_1 receptor-mediated impairment of passive avoidance performance and long-term potentiation in the hippocampus.** ***Biological & Pharmaceutical Bulletin*** **24:496-500, 2001.**

Abstract : We examined the effects of paeoniflorin on adenosine A_1 receptor-mediated memory disturbance in the mouse passive avoidance test and inhibition of long-term potentiation (LTP) in the rat hippocampal CA1 region. The pretraining administration of the selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) significantly impaired the retention performance determined 24 h after the training test. The intraperitoneal injections of paeoniflorin and the selective adenosine A_1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) significantly attenuated the deficit in retention performance caused by CPA. The in vitro studies revealed that adenosine (1 and 10 μM) dose dependently reduced both the population spike (PS) amplitudes and the tetanic stimulation-induced LTP in the hippocampus. DPCPX, at the concentration (0.1 μM) that had no effect on PS amplitudes or LTP induction, significantly reversed the suppressive effects of adenosine on both indices. Paeoniflorin also dose dependently reversed 10 μM adenosine-induced suppression of LTP but had no effect on PS reduced by adenosine. These results suggest that paeoniflorin ameliorates memory disruption mediated by adenosine A_1 receptor and that modulation of adenosine-mediated inhibition of LTP in the hippocampus may is implicated in its beneficial effect on learning and memory impairment in rodents.

3）**張紹輝，東田道久，村上孝寿，松本欣三，渡辺裕司：一過性虚血及び抗コリン薬スコポラミン誘発の学習障害に対する四物湯の改善作用及び処方解析.** ***Journal of Traditional Medicines*** **18:133-139, 2001.**

Abstract : In the previous paper, our group reported that Shimotsu-to improves scopolamine-induced spatial cognitive deficits in rats using an 8-arm radial maze. In the present study, we investigated the effect of Shimotsu-to on the impairment induced by transient cerebral ischemia in mice in passive avoidance performance. Shimotsu-to (6.0 g/kg, p.o.) administered one hour before operation showed the protective effect on impairment induced by the cerebral ischemia reperfusion. We also studied the efficacy of Shimotsu-to and its four crude fractions, Angelica Root, Cnidium Rihizome, Peony Root and Rehmannia Root on scopolamine-induced acquisition impairment. Shimotsu-to prevented scopolamine-induced acquisition impairment at dose of 1.5 and 6.0 g/kg (p.o.). Among the four constituents, Angelica and Peony prolonged the latency of step-down and decreased the number of errors in the retention trials at a high dose (1.5 mg/kg, p.o.). Whereas Rehmannia and Cnidium did not show any effect in the same performance. These results suggested that Angelica and Peony played an important role in the anti-amnesic effect of Shimotsu-to. The effect of Shimotsu-to on amnesia could not be explained entirely by the contribution of Angelica and Peony. The absence of any one of its constituents resulted in the loss of activity.

◇総　説　Reviews paper

1）**Guidotti A., Dong E., Matsumoto K., Pinna G., Rasmusson AM. and Costa E.: The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5α-dihydroprogesterone in psychiatric disorders. *Brain Research Reviews* 37: 110-115, 2001.**

Abstract : Allopregnanolone (3α,5α-TH PROG) and 5α-dihydroprogesterone (5α-DH PROG), the two most important neuroactive steroids synthesized in the brain, potently modulate neuronal activity by allosterically regulating GABA action at $GABA_A$ receptors or by changing specific $GABA_A$ receptor subunit gene expression, respectively. We recently reported [Proc. Natl. Acad. Sci. USA 95 (1998) 3239] that in patients with severe depression there is a decrease in the CSF levels of 3α,5α-TH PROG, which is normalized by treatment with drugs (i.e. fluoxetine) that improve psychopathology. The mechanism by which fluoxetine and other selective serotonin reuptake inhibitors normalize 3α,5α-TH PROG CSF levels appears to involve a direct stimulation of 3α-hydroxysteroidoxidoreductase (3α-HSD), an enzyme that catalyses the reduction of 5α-DH PROG into 3α,5α-TH PROG. Here, we propose the use of socially-isolated mice that have a downregulation of 3α,5α-TH PROG and of 5α-DH PROG expression to establish a model to study the behavioral consequences of this deficiency. After 4-6 weeks of isolation, these mice exhibit increased anxiety and aggressive behavior and also a decreased response to the administration of GABA-mimetic drugs. In these mice, the decrease in 3α,5α-TH PROG is selectively normalized by the use of fluoxetine in doses that reduce behavioral abnormalities. In addition, the expression of 5α-reductase Type I mRNA and protein was lower in socially-isolated mice than that in group-housed mice whereas 3α-HSD mRNA expression remained unchanged. The results of these studies may enable us to design drugs that specifically affect neurosteroidogenic enzymatic activities and may provide an efficacious treatment for the psychopathologies associated with psychiatric disorders.

◇学会報告　Scientific Presentations

1）野村浩明, 松本欣三, 高畑廣紀, ギドッチ アレッサンドロ, コスタ エルミニオ, 渡辺裕司: 内因性アロプレグナノロンによるマウス脳内 GABA-A 受容体機能の生理的制御. 第74回日本薬理学会年会, 2001, 3/21–23, 横浜.

2）田畑恵市, 松本欣三, 渡辺裕司: ラット海馬 CA1 領域におけるアデノシン誘発LTP抑制に対するペオニフロリンの効果. 第74回日本薬理学会年会, 2001, 3/21–23, 横浜.

3）松本欣三, 田畑恵市, 太田浩之, 赤澤康平, 市木裕之, 渡辺裕司: シンポジウム「漢方薬/生薬の薬理学的実証性と将来の展望」, Paeoniflorin の空間認知障害改善作用とその機序. 第74回日本薬理学会年会, 2001, 3/21–23, 横浜.

4）張紹輝, 村上孝寿, 松本欣三, 渡辺裕司, 小島暁: 一過性脳虚血及び抗コリン薬スコポラミン誘発の学習

記憶障害に対する釣藤散及び釣藤鈎の保護効果. 日本薬学会第121年会, 2001, 3/28-30, 札幌.

5）趙琦, 渡辺裕司, 村上孝寿, 東田道久, 松本欣三: 自然発症高血圧ラットにおける釣藤散の抗圧作用. 第18回和漢医薬学会大会, 2001, 8/18-19, 富山.

6）趙琦, 榊原巌, 渡辺裕司, 村上孝寿, 東田道久, 松本欣三: 自然発症高血圧ラットの血圧に対する釣藤鈎メタノールエキスおよびそのフェノール成分の作用. 第18回和漢医薬学会大会, 2001, 8/18-19, 富山.

7）張紹輝, 村上孝寿, 東田道久, 松本欣三, 榊原巌, 高山廣光, 相見則郎, 渡辺裕司: ミニシンポジウム「抗痴呆薬開発へのヒント」, 一過性脳虚血誘発のマウス学習障害に対する釣藤鈎の作用. 第18回和漢医薬学会大会, 2001, 8/18-19, 富山.

8）中島隆太郎, 東田道久, 渡辺裕司：Differential Display 法により単離した新規慢性脳虚血関連因子 vof-16, vof-21 の全長配列解析. 第52回日本薬理学会北部会, 2001, 10/6, 札幌.

◇招待講演 Invited lectures

1）Watanabe H.: Traditional medicine in Japan. International symposium for 10th Anniversary of WHO collaboration Center of Vietnam Institute of Traditional Medicine, 2001, 4/9, Hanoi.

2）Matsumoto K. : Anti-stress effects of Vietnamese ginseng and majonoside-R2: a putative mechanism of actions. XXX Congresso Nazionale della Societa Italiana di Farmacologia, 2001, 5/30-6/2, Genova.

3）Watanabe H.: Pharmacological study on anti-dementia effects of Choto-san and Shimotsu-to in experimental animals. Symposium on the Role of Traditional Medicine in the 21st Century, 2001, 8/20-21, Toyama.

4）Watanabe H.: Pharmacological study on anti-dementia effect of traditional medicines. International Symposium: In Commemoration of the 30th Anniversary of the Foundation of Kyung Hee University Medical Center and the 20th Anniversary of the International Day of Peace: The Cooperation of East-West Medicine in the 21st Century, 2001, 10/5-6, Seoul.

5）Watanabe H.: Traditional Medicine in Japan: Past, Present and Future. The 8th International Symposium on Traditional Medicine in Toyama (2001), 2001, 10/11-12, Toyama.

6）Watanabe H.: The Future of Traditional Medicines. 18th Annual Research Meeting in Pharmaceutical Sciences at Chulalongkorn University, 2001, 12/11-12, Bangkok.

◇その他 Others

1）渡邉裕司: 漢方方剤の中枢薬理作用の研究. 第18回和漢医薬学会大会(会長講演), 2001, 8/18-19, 富山.

◇共同研究 Co-operative researches

1）相見則郎, 高山廣光：千葉大学薬学部,「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－

2）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：ベトナム伝統医学研究所,「ベトナム人参の薬理作用の研究」1994, 4－

3）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 Acquisition of research funds

1）文部科学省科学研究費, 萌芽的研究（代表：渡辺裕司)「老年痴呆モデルラットの対光縮瞳反射に関する研究」 170万

2）文部科学省科学研究費, 基盤研究Ｂ（２）(代表：渡辺裕司)「慢性脳循環障害モデル動物の白質および神経細胞変性発症機序の解析」 750万

3）文部科学省科学研究費, 基盤研究Ｂ（２）(代表：東田道久)「慢性虚血ラット脳中で発現変化する新規単離因子の生理機能・発現制御機構の解明」 250万

4）受託研究費，小野薬品工業（渡辺裕司）「一過性脳虚血マウスの痴呆モデルとしての意義と薬物作用の研究」 250万
5）平成13年度科学技術振興調整経費（渡辺裕司）「脳血管性痴呆，高血圧などの生活習慣病を予防・治療する漢方方剤及び生薬類の研究」1100万
6）富山第一銀行奨学財団研究費，（代表：東田道久）「精神疾患治療効果を有する和漢薬の作用機序としての脳内遺伝子発現」 50万
7）平成13年度創造開発研究費（代表：松本欣三）「脳内神経ステロイド系障害に対する天然薬物作用の研究」 142万

◇研究室在籍者　Research Members

学部4年生：神野雄一，森繁亮
大学院前期2年：中島隆太郎
大学院後期1年： Pramote Mahakunakorn
大学院後期2年：姜太炫，Monrudee Sukma
大学院後期3年：張紹輝
研究支援推進員： 中村政美，趙琦
外国人特別研究学生：
Sumittra Gomonchareonsiri（日本国際教育協会，チュラロンコン大学，2000, 9–2001, 9)
Preecha Boonchoong（日本国際教育協会及びタイ王立教育協会，マヒドン大学，2001, 5/10–12/19）
Luis Miguel Melendez Queija（松下国際財団，2001, 4/5–2002, 3/31）
外国人客員研究員：
Dr. Wantana Reanmongkol（プリンスオブソンクラ大学，2001, 2/2-2/24）
Dr. Ghazi Hussein（Khartoum 大学教育助手，2002, 4/1–2003, 3/31）
相　婷　博士（瀋陽薬科大学講師，2002, 4/1–2003, 3/31）
短期訪問研究者(拠点大学方式による学術交流事業：研究者交流)：
Dr. Opa Vajragupta（マヒドン大学，2001, 10/8–10/23）
Dr. Sunibhond Pummangura（チュラロンコン大学，2001, 10/8–10/16）
Dr. Poj Kulvanich（チュラロンコン大学，2001,10/8–10/16）

◇学位取得者　Academic degrees and Thesis

修士 Master：
野村浩明　内因性神経活性ステロイド allopregnanolone の生理的役割に関する研究
博士 Ph.D.：
田畑恵市　芍薬成分ペオニフロリンの学習行動障害改善作用に関する研究