| | | | | | |
|---|---|---|---|---|---|
| Fukui N,<br>Inoue Y,<br>**Someya T** | | | | | |
| Sawamura K,<br>Suzuki Y,<br>Fukui N,<br>Sugai T,<br>**Someya T** | Gender differences in prolactin elevation induced by olanzapine in Japanese drug-naive schizophrenic patients | Prog Neuropsychopharmacol Biol Psychiatry | 30(8) | 1511-1514 | 2006 |
| Ozdemir V,<br>Gunes A,<br>Dahl ML,<br>Scord MG,<br>Williams-Jones B,<br>**Someya T** | Could endogenous substrates of drug-metabolizing enzymes influence constitutive physiology and drug target responsiveness? | Pharmacogenomics | 7(8) | 1199-1210 | 2006 |
| Yasui-Furukori N,<br>Saito M.<br>Nakagami T,<br>Kaneda A,<br>Tateishi T,<br>**Kaneko S** | Association between Multidrug Resistance 1 (MDR1) gene polymorphism and therapeutic response to bromperidol in schizophrenic patients:<br>A preliminary study | Prog Neuropsychopharmacol Biol Psychiatry | 30 | 286-291 | 2006 |
| Yasui-Furukori N,<br>**Kaneko S** | Digitalis intoxication induced by paroxetine co-administration | Lancet | 367 (9512) | 788 | 2006 |
| Niioka T,<br>Yasui-Furukori N,<br>Uno T,<br>Sugawara K,<br>**Kaneko S,**<br>Tateishi T | Identification of the time-point to give a plasma lansoprazole copncentration that adequately reflected area under the concentration-time curve. | Ther Drug Monit | 28 | 321-325 | 2006 |
| Saitoh, A.,<br>Yamada M.,<br>**Yamada M,**<br>Kobayashi' S,<br>Hirose N,<br>Honda K<br>Kamei J | ROCK inhibition produces anxiety-related behaviors in mice | J Psychopharmacology | 188 | 1-11 | 2006 |
| Tanaka S,<br>Yamada M,<br>Kitahara S,<br>Higuchi T,<br>Honda K,<br>Kamijima K,<br>**Yamada M** | Induction of Neuroserpin Expression in Rat Frontal Cortex after Chronic Antidepressant Treatment and Electroconvulsive Treatment | Jpn J Neuropsychopharmacol | 26(1) | 51-56 | 2006 |
| Saitoh A,<br>Hirose N,<br>Yamada M,<br>**Yamada M,**<br>Nozaki C,<br>Oka T,<br>Kamei J | Changes in Emotional Behavior of Mice in the Hole-Board Test After Olfactory Bulbectomy | J Pharmacol Sci | 102 | 377-386 | 2006 |
| Ozdemir V,<br>Williams-Jones B,<br>Cooper DM,<br>**Someya T,**<br>Godard B | Mapping translational research in the age of theragnostics:<br>from molecular markers to personalized drug therapy | Pharmacogenomics | 8(2) | 177-185 | 2007 |
| Ozdemir V,<br>Williams-Jones B,<br>Graham JE,<br>Preskorn SH,<br>Gripeos D, | Asymmetry in scientific method and limits to cross-disciplinary dialogue: Toward a shared language and science policy in pharmacogenomics and human disease genetics. | J Invest Med | 55(3) | 130-141 | 2007 |

| | | | | | |
|---|---|---|---|---|---|
| Glatt SJ,<br>Friis RH,<br>Reist C,<br>Szabo S,<br>Lohr JB,<br>**Someya T**: | | | | | |
| Fukui N,<br>Suzuki Y,<br>Sawamura K,<br>Sugai T,<br>Watanabe J,<br>Inoue Y,<br>**Someya T** | Dose-dependent effects of the 3435 C>T genotype of ABCB1 gene on the steady-state plasma concentration of fluvoxamine in psychiatric patients | Ther Drug Monit. | 29(2) | 185-189 | 2007 |
| Yasui-Furukori N,<br>Saito M,<br>Inoue Y,<br>Niioka T,<br>Sato Y,<br>Tsuchimine S,<br>**Kaneko S** | Terbinafine increases the plasma concentration of paroxetine after a single oral administration of paroxetine in healthy subjects | Eur J Clin Pharmacol | 63 | 51-56 | 2007 |
| Yasui-Furukori N, Saito M, Niioka T, Inoue Y, Sato, **Kaneko S** | Effect of itraconazole on pharmacokinetics of paroxetine: The role of gut transporters | Ther Drug Monit | 29(1) | 45-48 | 2007 |
| Iwashima K,<br>Yasui-Furukori N,<br>Kaneda A,<br>Saito, M.,<br>Nakagami, T.,<br>Tsuchimine, T.,<br>**Kaneko, S** | No association between CYP2D6 polymorphisms and personality trait in Japanese | Br. J Clin Pharmacol | 29(1) | 96-99 | 2007 |
| Yasui-Furukori N,<br>Saito M,<br>Nakagami T,<br>Niioka T,<br>Sato Y,<br>Fujii A,<br>**Kaneko S** | Different serum concentrations of valproic acid at steady state between two sustained-release formulations | Psychiatry Clin Neurosci | 61(3) | 308-312 | 2007 |
| Yasui-Furukori N,<br>Tsuchimine S,<br>Saito M,<br>Nakagami T,<br>Sato Y,<br>**Kaneko S** | Association between Multidrug Resistance 1 (MDR1) gene polymorphisms and plasma concentration of prolactin during risperidone treatment in schizophrenic patients | Progress in Neuropsychopharmacology & Biological Psychiatry | 31(6) | 1230-1234 | 2007 |
| Yasui-Furukori N,<br>Kaneda A,<br>Iwashima K,<br>Saito, M.,<br>Nakagami, T.,<br>Tsuchimine, T.,<br>**Kaneko, S** | Association between CYP2C19 polymorphisms and Harm Avoidance in Japanese | Am. J Med Genetics | 144B | 724-727 | 2007 |
| Yoshida K,<br>Higuchi H,<br>Kamata M,<br>Takahashi H,<br>Inoue K,<br>Suzuki T,<br>Itoh K,<br>**Ozaki N** | The G196A polymorphism of the brain-derived neurotrophic factor gene and the antidepressant effect of milnacipran and fluvoxamine | J Psychopharmacol | 21(6) | 650-656 | 2007 |

| | | | | | |
|---|---|---|---|---|---|
| Saito S,<br>Takahashi N,<br>Ishihara R,<br>Ikeda M,<br>Suzuki T,<br>Kitajima T,<br>Yamanouchi Y,<br>Iwata N,<br>**Yamada M**,<br>Yoshida K,<br>Inada T,<br>**Ozaki N**: | Association Study between Vesicle-Associated Membrane Protein 2 Gene Polymorphisms and Fluvoxamine Response in Japanese Major Depressive Patients | Neuropsychobiology | 54(4) | 226-230 | 2007 |
| Kamei J,<br>Hirose N,<br>Oka T,<br>Miyata S,<br>Saitoh A,<br>**Yamada M** | Effects of Methylphenidate on the Hyperemotional Behavior in Olfactory Bulbectomized Mice by Using the Hole-Board Test | J Pharmacol Sci | 103 | 175-180 | 2007 |
| Matsuzawa D,<br>Hashimoto K,<br>Miyatake R,<br>Shirayama Y,<br>Shimizu E,<br>Maeda K,<br>Suzuki Y,<br>Mashimo Y,<br>Sekine Y,<br>Inada T,<br>**Ozaki N**,<br>Iwata N,<br>Harano M,<br>Komiyama T,<br>**Yamada M**,<br>Sora I,<br>Ujike H,<br>Hata A,<br>Sawa A,<br>Iyo M | Identification of Functional Polymorphisms in the Promoter Region of the Human PICK1 Gene and Their Association With Methamphetamine Psychosis | Am J Psychiatry | 164 | 1105-1114 | 2007 |
| Yoshida K,<br>Higuchi H,<br>Takahashi H,<br>Kamata M,<br>Sato K,<br>Inoue K,<br>Suzuki T,<br>Itoh K,<br>**Ozaki N** | Influence of the tyrosine hydroxylase val81met polymorphism and catechol-O-methyltransferase val158met polymorphism on the antidepressant effect of milnacipran | Hum Psychopharmacol | | | in press |
| Ji X,<br>Takahashi N,<br>Saito S,<br>Ishihara R,<br>Maeno N,<br>Inada T,<br>**Ozaki N** | Relationship between three serotonin receptor subtypes (HTR3A, HTR2A and HTR4) and treatment-resistant schizophrenia in the Japanese population | Neurosci Lett | | | in press |
| Tanaka M,<br>Kobayashi D,<br>Murakami Y,<br>**Ozaki N**,<br>Suzuki T,<br>Iwata N, | Genetic polymorphisms in the 5-hydroxytryptamine type 3B receptor gene and p aroxetine-induced nausea | Int J Neuropsychopharmacol | | 1-7 | in press |

| | | | | | |
|---|---|---|---|---|---|
| Haraguchi K,<br>Ieiri I,<br>Kinukawa N,<br>Hosoi M,<br>Ohtani H,<br>Sawada Y,<br>Mine K | | | | | |
| Masui T,<br>Hashimoto R,<br>Kusumi I,<br>Suzuki K,<br>Tanaka T,<br>Nakagawa S,<br>Suzuki T,<br>Iwata N,<br>**Ozaki N**,<br>Kato T,<br>Takeda M,<br>Kunugi H,<br>Koyama T | A possible association between missense polymorphism of the breakpoint cluster region gene and lithium prophylaxis in bipolar disorder | Prog Neuropsychopharmacol Biol Psychiatry | | | in press |
| 鈴木雄太郎,<br>福井直樹,<br>**染矢俊幸** | 遺伝子多型と抗うつ薬の治療反応性および副作用 | カレントテラピー | 23(1) | 73-77 | 2005 |
| 鈴木雄太郎,<br>**染矢俊幸** | 卒後臨床研修で習得すべき精神科薬物療法 | 精神科臨床サービス | 5(1) | 82-86 | 2005 |
| **近藤毅** | 精神科治療のテーラーメイド化を進めるうえでの基本コンセプト | 精神科 | 7 | 405-408 | 2005 |
| 須貝拓朗,<br>澤村一司,<br>**染矢俊幸** | 抗精神病薬による注目すべき有害事象 -非定型抗精神病薬を中心に- | 臨床精神薬理 | 9(3) | 423-429 | 2006 |
| 福井直樹,<br>**染矢俊幸** | 抗うつ薬血中濃度の今日的意義 | 臨床精神薬理 | 9(4) | 593-599 | 2006 |
| 鈴木雄太郎,<br>澤村一司,<br>**染矢俊幸** | うつ病及び統合失調症の薬物治療反応性に関する分子薬理遺伝学的研究 | 精神神経学雑誌 | 108(6) | 633-641 | 2006 |
| **山田光彦**,<br>山田美佐,<br>高橋弘,<br>丸山良亮 | 遺伝子から探る新規抗うつ薬の開発 | 日本薬理学雑誌 | 128(1) | 19-22 | 2006 |
| 福井直樹,<br>鈴木雄太郎,<br>**染矢俊幸** | 薬物動態および薬力学(臨床効果,副作用)の予測，オーダーメイド薬物治療 | 月刊 Mebio | 24(2) | 108-117 | 2007 |
| 渡邉純蔵,<br>鈴木雄太郎,<br>澤村一司,<br>須貝拓朗,<br>福井直樹,<br>小野信,<br>**染矢俊幸** | 精神疾患とメタボリックシンドローム | 臨床精神薬理 | 10(3) | 387-393 | 2007 |
| 小野信,<br>鈴木雄太郎,<br>**染矢俊幸** | 選択的セロトニン再取り込み阻害薬の治療反応性および副作用出現の予測に関する分子薬理遺伝学的研究 | 脳と精神の医学 | 18(1) | 27-33 | 2007 |

| | | | | | |
|---|---|---|---|---|---|
| 丸山良亮,<br>山田光彦 | TREK-1 カリウムチャネル:<br>抗うつ薬創出の新たなターゲット | 日本神経精神薬理学雑誌 | 27 | 147-151 | 2007 |

# Ⅳ. 研究成果の刊行物・別刷

# The Expression of Synaptic Vesicle Proteins after Chronic Antidepressant Treatment in Rat Brain

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**Summary.** The biological basis for the therapeutic mechanisms of depression are still unknown. We have previously performed EST analysis and identified some common biological changes induced after chronic antidepressant treatment as antidepressant related genes/ESTs : ADRG#1-707. Then, we developed our original cDNA microarray on which ADRG#1-707 were spotted, for rapid secondary screening of candidate genes as the novel therapeutic targets. With this microarray, we found that the expression of some of the ADRGs were related to neurotransmiter release and located on synaptic vesicle. Indeed, VAMP2/synaptobrevin, cysteine string protein, synapsin I, Rab-1A and Rab-3B were induced after chronic sertraline treatment in rat frontal cortex. Western blot analysis also demonstrated the induction of these ADRGs at protein levels after chronic treatment with imipramine and sertraline. In addition, synaptophysin and secretogranin II, often used as a marker protein for small synaptic vesicle or large dense core granule were significantly increased after chronically treatment with antidepressants. On the other hand, the expression of SNAP-25 and syntaxin-1, which are used as markers for synapse and make a SNARE-complex with VAMP2, were not affected by these treatments. These results suggested that the number of synaptic vesicles, but not the number of synapses, was increased after chronic antidepressant treatment. The synaptic vesicles and proteins may be a new target molecular system for antidepressant.

**Key words.** Depression, antidepressant, microarray, synaptic vesicle, SNARE complex

## 1 Introduction

It has been demonstrated that typical antidepressants acutely inhibit the monoamine reuptake in nerve terminals resulting in significant increase in synaptic concentrations of monoamines, noradrenaline or serotonin. However, there is a latency period of several weeks before the onset of clinical effect of antidepressants. There are several preclinical investigation shown the delayed action of antidepressants on mood, motivation and cognition is not linked to their primary mechanism of action but rather to the development of various modifications (Duman and Vaidya 1998). Hyman and Nestler proposed an "initiation and adaptation" model to describe the drug-induced neural plasticity associated with the long-term actions of antidepressants in the brain (Hyman and Nestler 1996). However, the detailed mechanisms underlying such drug-induced adaptive neuronal changes are as of yet unknown. The delay of clinical effect from antidepressants could be the result of indirect regulation of neural signal transduction systems or changes at the molecular level by an action on gene transcription following chronic treatment. Indeed, there are selective effects of antidepressants on specific immediate early genes and transcription factors. These molecules activate or repress genes encoding specific proteins by binding to a regulating element of DNA. These functional proteins may be involved in critical steps in mediating treatment-induced neural plasticity. Therefore, we demonstrated that certain novel candidate genes and molecular systems may underlie the mechanism of action of antidepressants.

## 2 EST Analysis and Fabrication of the Original cDNA Microarray for Antidepressant Research

We have performed expressed-sequence tag (EST) analysis to identify some common biological changes induced after chronic treatment of two different classes of antidepressants, imipramine (a tricyclic antidepressant) and sertraline (a serotonin selective reuptake inhibitor, SSRI). Identification of quantitative changes in gene expression that occur in the brain after chronic antidepressant treatment can yield novel molecular machinery responsible for therapeutic effect of antidepressant. Until now, we have molecularly cloned 707 cDNA fragments which were named them antidepressant related genes, ADRG from rat frontal cortex, hippocampus and hipothalamus (Yamada et al. 1999). More recently, for high throughput secondary screening of candidate genes, each of the ADRGs were spotted in duplicate onto glass slides to develop our original microarray, ADRG microarray. After hybridization with samples obtained from sertraline treated rat frontal cortex and normalization of the signals for both negative and positive controls, we have identified several interesting candidate genes and ESTs on the ADRG microarray that showing increased or decreased expression compared from control.

## 3 New Candidate Molecular Systems in Depression Research

We found some of the candidate molecules and molecular systems with this ADRG microarray. Interestingly, the expression of some of the ADRGs related to neurotransmiter release and located on synaptic vesicles were induced after chronic treatment with sertraline in rat frontal cortex (Fig. 1). We previously reported that the expression of ADRG55, identified as cysteine string protein (CSP), was induced after chronic antidepressant treatment (Yamada et al. 2001). CSP is localized to synaptic vesicle membranes and modulates the activity of presynaptic calcium channels, resulting in neurotransmitter release at the nerve terminal in the central nervous system (Gundersen et al. 1995). In addition, we have also demonstrated that the expression of ADRG14, identified as vesicle associated membrane protein VAMP2/ synaptobrevin, was induced after chronic antidepressant treatment (Yamada et al. 2002). VAMP2/ synaptobrevin is a key component of the synaptic vesicle docking/fusion machinery that forms the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) complex (Weis and Scheller 1998). More recently, we identified some more ADRGs related to neurotransmiter release and located on synaptic vesicles, including synapsin I, Rab-1A and Rab-3B. Synapsin I is an actin-binding protein that localized on the cytoplasmic face of small synaptic vesicles and inhibits neurotransmitter release, an effect that is abolished upon its phosphorylation by $Ca^{2+}$/calmodulin-dependent protein kinase II. Rab protein is low molecular weight GTP-binding protein of the Ras superfamily of GTPases. Rab protein is involved in intracellular membrane fusion reactions located in cytoplasmic face of organelles and vesicles. The synapsin I, Rab-1A and Rab-3B were induced after chronic antidepressant treatment in rat frontal cortex, when determined by ADRG microarray.

Western blot analysis also demonstrated the induction of these synaptic vesicle proteins after chronic treatment with imipramine and sertraline in rat frontal cortex. These results indicate that two possibilities i) the number of synaptic vesicles is increased, ii) the number of synapses is increased after chronic antidepressant treatment. To investigate the first possibility, the expression of synaptophysin and secretogranin II were determined by Western blot analysis. Then, to investigate the second possibility, the expression of SNAP-25 and syntaxin-1, which are used as markers for synapse and make a SNARE-complex with VAMP2, were determined. Interestingly, the expression of both synaptophysin (a marker protein for small synaptic vesicles) and secretogranin II (a marker protein for large dense core granules) were significantly increased after chronic treatment with antidepressants. On the other hand, the expression of SNAP-25 and syntaxin-1 were unaffected by these treatments. These results strongly suggested that the number of synaptic vesicles, but not the number of synapses, was increased after chronic antidepressant treatment.

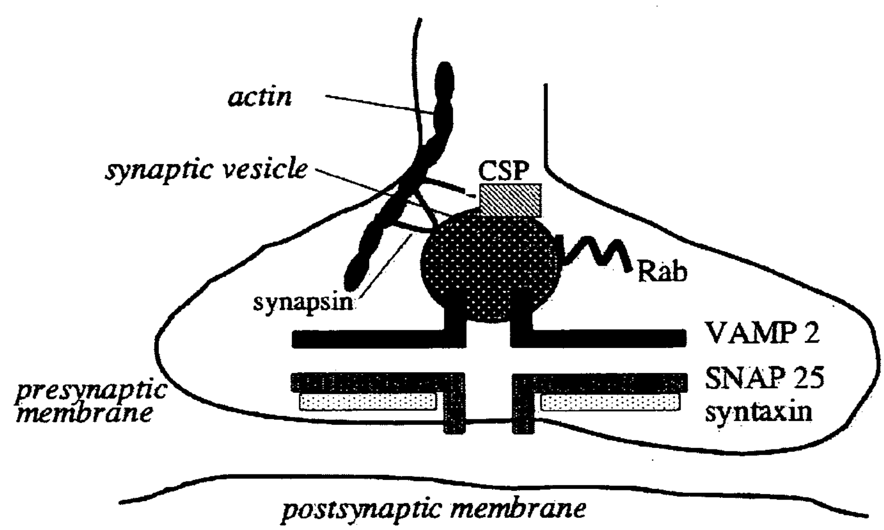


**Fig. 1.** Synaptic vesicle proteins identified as ADRG and related proteins. The expression of some of the ADRGs related to neurotransmiter release and located on synaptic vesicle including VAMP2/synaptobrevin, cysteine string protein (*CSP*), synapsin I, Rab-1A and Rab-3B were induced after chronic sertraline treatment in rat frontal cortex determined by ADRG microarray

There are several articles that reporting the change of synaptic protein expression, activity or phosphorylation affected by antidepressant treatments (Popoli et al. 1995). Further, long-term treatment of hippocampal slice cultures with brain-derived neurotrophic factor (BDNF) that is one of the key molecule induced by antidepressant treatment (D'Sa and Duman 2002) increased the number of docked vesicles, but not that of reserve pool vesicles at CA1 excitatory synapses. BDNF also increased the levels of vesicle proteins synaptophysin, synaptobrevin, and synaptotagmin, without affecting the presynaptic membrane proteins syntaxin and SNAP-25, or the vesicle-binding protein synapsin-I (Tartaglia et al. 2001). Taken together, these finding may suggest a link between the modulation of synaptic vesicle proteins and the therapeutic mechanisms of antidepressants.

## 4 Conclusion

In the present study, we have demonstrated that the synaptic vesicles and proteins may play a role in the therapeautic molecular systems of antidepressant treatment. These alterations of the expression pattern of synaptic vesicle proteins may also be associated with neural plasticity including modifications in neural connectivity, and modulation of synaptic vesicle density that occur during antidepressant treatment. Here, we propose that the changes in neural plasticity are implicated in the adaptive mechanisms underlie the delayed onset of therapeutic action of antidepressants. Our results may contribute to a novel model for the therapeutic mechanism of depression and new molecular targets for the development of therapeutic agents.

## References

D'Sa C, Duman RS (2002) Antidepressants and neuroplasticity. Bipolar Disord 4: 183-194

Duman RS, Vaidya VA (1998) Molecular and cellular actions of chronic electroconvulsive seizures. J Ect 14: 181-193

Gundersen CB, Mastrogiacomo A, Umbach JA (1995) Cysteine-string proteins as templates for membrane fusion: models of synaptic vesicle exocytosis. J Theor Biol 172: 269-277

Hyman SE, Nestler EJ (1996) Initiation and adaptation: a paradigm for understanding psychotropic drug action. Am J Psychiatry 153: 151-162

Popoli M, Vocaturo C, Perez J, Smeraldi E, Racagni G (1995) Presynaptic Ca2+/calmodulin-dependent protein kinase II: autophosphorylation and activity increase in the hippocampus after long-term blockade of serotonin reuptake. Mol Pharmacol 48: 623-629

Tartaglia N, Du J,Tyler WJ, Neale E, Pozzo-Miller L, Lu B (2001) Protein synthesis-dependent and -independent regulation of hippocampal synapses by brain-derived neurotrophic factor. J Biol Chem 276: 37585-37593

Weis WI, Scheller RH (1998) Membrane fusion. SNARE the rod, coil the complex. Nature 395: 328-329

Yamada M, Yamada M, Kiuchi Y, Nara K, Kanda Y, Morinobu S, Momose K, Oguchi K, Kamijima K, Higuchi T (1999) Identification of a novel splice variant of heat shock cognate protein 70 after chronic antidepressant treatment in rat frontal cortex. Biochem Biophys Res Commun 261: 541-545

Yamada M, Takahashi K, Tsunoda M, Nishioka G, Kudo K, Ohata H, Kamijima K, Higuchi T, Momose K, Yamada M (2002) Differential expression of VAMP2/synaptobrevin-2 after antidepressant and electroconvulsive treatment in rat frontal cortex. Pharmacogenomics J 2: 377-382

Yamada M, Yamada M, Yamazaki S, Nara K, Kiuchi Y, Ozawa H, Yamada S, Oguchi K, Kamijima K, Higuchi T, Momose K (2001) Induction of cysteine string protein after chronic antidepressant treatment revealed by ADRG microarray. Neurosci Lett 301: 183-186

# Identification of Molecular Systems Responsible for the Therapeutic Effect of Antidepressant

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**Summary.** Although blockade by antidepressants of monoamine uptake into nerve endings is one of the cornerstones of the monoamine hypothesis of depression, there is a clear discrepancy between the rapid effects of antidepressants in increasing synaptic concentrations of monoamine and the lack of immediate clinical efficiency of antidepressant treatment. Pharmacogenomics, functional genomics and proteomics are powerful tools that can be used to identify genes/ESTs or molecular systems affected by antidepressants. Using a differential cloning strategy, we and other groups have isolated genes that are differentially expressed in the brain after chronic antidepressant treatment. Some of these candidate genes may encode functional molecular systems or pathways induced by chronic antidepressant treatment. Defining the roles of these molecular systems in drug-induced neural plasticity is likely to transform the course of research on the biological basis of depression. Such detailed knowledge will have profound effects on the diagnosis, prevention, and treatment of depression.

**Key words.** Depression, antidepressant, differential cloning, system biology

## 1 Introduction

Depression is one of the major psychiatric diseases; represent abnormality of emotional, cognitive, autonomic and endocrine functions. Anti- depressants are very effective agents for the prevention and treatment of depression, and have been used clinically for more than 50 years. Although the therapeutic action of these antidepressants most likely involves the regulation of serotonergic and noradrenergic signal transduction pathways, to date, no consensus has been reached concerning the precise molecular and cellular mechanism of action of these drugs. Many antidepressants acutely regulate monoaminergic signal transduction within a few hours of initial treatment. However, at the same time, the onset of the clinical effect of these drugs lags by several weeks. A satisfying explanation for the discrepancy in the acute increase of synaptic monoamines and delayed clinical effect remains elusive. Consequently, the monoamine hypothesis does not fully explain this clear discrepancy. Novel biological approaches beyond the "monoamine hypothesis" are definitely expected to cause paradigm shifts in the future of depression research. In this article, we demonstrated that certain novel candidate molecular systems might underlie the mechanism of action of antidepressants.

## 2 The Delayed Clinical Effects and Changes in Gene Expression Elicited by Antidepressant in the Brain

To advance our understanding of the therapeutic actions of anti- depressants, we must now extend our efforts beyond theories based on the simple pharmacology of the synapse. This new effort must seek a deeper understanding of cellular and molecular neurobiology as well as examine the architecture and function of relevant neural systems. Many now believe that changes in brain gene expression, which are elicited after chronic antidepressant treatment, might underlie the drug-induced neural plasticity associated with the long-term actions of antidepressants in the brain and their clinical effects.

On the other hand, there are several preclinical investigations shown that the delay of clinical effect from antidepressants could be the result of indirect regulation of neural signal transduction systems or changes at the molecular level by an action on gene transcription following chronic treatment. Indeed, there are selective effects of antidepressants on specific immediate early genes and transcription factors including c-fos, zif268, NGFI-A and the phosphorylation of CRE binding protein. These molecules activate or repress genes encoding specific proteins by binding to a regulating element of DNA. These functional proteins may be involved in critical steps in mediating treatment-induced neural plasticity (see review by Yamada et al., 2002).

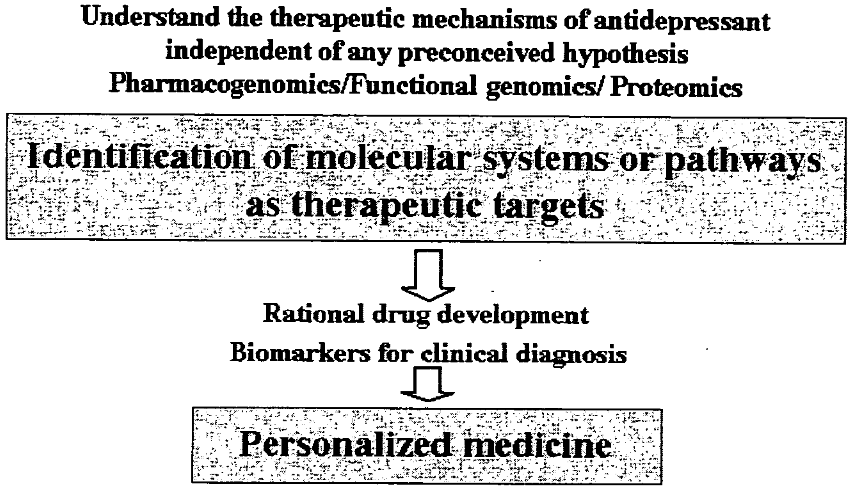


**Fig. 1.** Pharmacogenomics, Functional genomics and Proteomics are powerful tools that can be used to identify neuronal systems or pathways affected by antidepressants

Recent developments in molecular neurobiology provide new conceptual and experimental tools to investigate, and facilitate understanding of the mechanisms by which antidepressants produce long-lasting alterations in brain function. The emerging techniques and powerful tools derived from the relatively new subfields of genomics and proteomics hold great promise for the identification of genes and gene products that are altered by chronic antidepressant treatment or other effective therapeutic manipulations, such as electroconvulsive treatment (ECT). Using a differential cloning strategy, we and other groups have isolated genes that are differentially expressed in the brain after chronic antidepressant treatment. Independent of any preconceived hypothesis, these genes and proteins have been implicated in a physiological or pathophysiological process. Defining the roles of the candidate systems in antidepressant-induced neural plasticity is likely to transform the course of research on the biological basis of mood disorders, leading to develop a personalized medicine (Fig.1). Such detailed knowledge will have profound effects on the diagnosis, prevention, and treatment of depression.

## 3 New Candidate Molecular Systems Responsible for the Therapeutic Effect of Antidepressant

Many now believe that changes in brain gene expression, which are elicited after chronic antidepressant treatment, might underlie the drug-induced neural plasticity associated with the long-term actions of antidepressants in the brain and their clinical effects. Here, we introduce three of the new candidate molecular systems in antidepressant research.

### 3.1 Adult Neurogenesis in the Hippocampus

Although depression involves many psychological and social factors, it also represents a biological process: the effects of repeated exposure to stress on a vulnerable brain. Preclinical and clinical research has focused on the interactions between stress and depression and their effects on the hippocampus (Duman et al., 1999). The hippocampus is one of several brain regions that, when exposed to stressful stimuli, can contribute to the emotional, cognitive, and vegetative abnormalities found in depressed patients. This region of the brain is also involved in the feedback regulation of the hypothalamus pituitary adrenal axis, the dysfunction of which is associated with depression. Recent studies suggest that stress-induced atrophy and loss of hippocampal neurons may contribute to the pathophysiology of depression. Interestingly, hippocampal volume is decreased in patients with stress-related psychiatric illnesses, including depression and post-traumatic stress disorder (Sapolsky and Duman, 2000; Sheline et al., 1996).

In vitro and in vivo data provide direct evidence that brain-derived neurotrophic factor (BDNF) is one of the key mediators of the therapeutic response to antidepressants (D'Sa and Duman, 2002). BDNF promotes the differentiation and survival of neurons during development and in the adult brain, as well as in cultured cells. Stress decreases the expression of BDNF, and reduced levels could contribute to the atrophy and compromised function of stress-vulnerable hippocampal neurons. In contrast, antidepressant treatment increases the expression of BDNF in the hippocampus, and could thereby reverse the stress-induced atrophy of neurons or protect these neurons from further damage (Duman, 1998; Duman et al., 1997). These findings have resulted in the development of a novel model of the mechanism of antidepressant action and have suggested new targets for the development of therapeutic agents.

While hippocampal volume can decrease in disease, the hippocampus is also one of only a few brain regions where the production of neurons normally occurs throughout the lifetime of several species of animals, including humans (Eriksson et al., 1998). Hippocampal neurogenesis is influenced by several environmental factors and stimuli (Gould and Tanapat, 1999; Nilsson et al., 1999; van Praag et al., 1999). For example, both acute and chronic stress cause decreases in cell proliferation. On the other hand, administration of several different classes of antidepressant, as opposed to non-antidepressant, agents increases the number of BrdU-labeled cells, indicating that this is a common and selective action of antidepressants (Malberg et al., 2000). In addition, recent evidence indicates that electroconvulsive seizures (an animal model of ECT in humans) can also enhance neurogenesis in rat hippocampus (Hellsten et al., 2002). These findings raise the possibility that increased cell proliferation and increased neuronal number may be a mechanism by which antidepressant treatment mitigates stress-induced atrophy and loss of hippocampal neurons, and thus may contribute to the therapeutic actions of antidepressant treatment. Furthermore, increased formation of new neurons in the hippocampus related to antidepressant treatment may lead to altered expression of genes specifically expressed in immature neurons. Therefore, observed changes in gene expression may reflect alterations in cell composition of the tissue rather than changes in individual neurons.

### 3.2 Vesicular Transport/Exocytotic Machinery

In our laboratory, we employed the RNA fingerprinting technique, a modified differential display PCR, to identify biochemical changes induced by chronic antidepressant treatments. To date, we have cloned several cDNA candidates as ESTs from rat frontal cortex and hippocampus. Some of these candidate cDNAs should be affected by antidepressants and are thus named antidepressant related genes (ADRGs). Among these ADRGs, we previously demonstrated that a unique cysteine-rich protein, called cysteine string protein (CSP), is clearly elevated in rat brain after chronic antidepressant treatment (Yamada et al., 2001). In rat brain, CSP interacts with VAMP2 in synaptic vesicle membranes and modulating the activity of presynaptic calcium channels, resulting in neurotransmitter release at the nerve terminal. Considerable evidence indicates that VAMP-2 is a key component of the synaptic vesicle transport/docking/fusion machinery that forms the SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) complex. Fusion of vesicles with the plasma membrane leads to exocytosis, which mediates the release of neurotransmitter into the synapse. Recently, we demonstrated a significant increase of both VAMP2 mRNA and protein levels in rat frontal cortex after chronic treatment with antidepressant and repeated ECT (Yamada et al., 2002). In this context, pharmacological modulations of CSP and VAMP2 expressions would also be predicted to alter neurotransmitter release. Interestingly, the work of others shows that acute and chronic administration of antidepressants diminishes the release of glutamate and aspartate, and inhibits veratridine-evoked 5-HT release (Golembiowska and Dziubina, 2000).

On the other hand, post-mortem depressive suicide brain samples were investigated to test the hypothesis that the regulation of SNARE proteins could be abnormal in depression (Honer et al., 2002). Interestingly, the immunoreactivity of VAMP2 was increased in depressive group. Further, the correlation between VAMP2 and other SNARE protein or synaptophysin were remarkably weak, and in some cases clearly non-significant. Of course, there were limitation of the availability of tissue for investigation and drug treatment history; the authors concluded that the abnormalities of SNARE complex could represent a molecular substrate for abnormalities of neural connectivity in depression.

Popoli and his fellows have demonstrated that the long-term treatment with antidepressants induced presynaptic CaM Kinase II activity, one of the kinases present involved in the modulation of transmitter release. Further, phosphorylation of synapsin I and synaptotagmin, the presynaptic substrates of CaM Kinase II were also increased after these treatments (Celano et al., 2003). In addition, in the amygdala of rats that received daily treatment with the TCA imipramine for 3 weeks, the gene encoding a mutation suppressor for the Sec4-8 yeast (Mss4) transcript was overexpressed (Andriamampandry et al., 2002). Mss4 protein has the properties of a guanine nucleotide exchange factor, and interacts with several members of the Rab family implicated in $Ca^{2+}$-dependent exocytosis of neurotransmitters. Interestingly, Mss4 transcripts were specifically downregulated in the hippocampus and amygdala of rats after exposure to chronic, mild stress. These findings suggest that gene expression- dependent alterations of neuronal transmitter release may be an important component of the pharmacological action of antidepressants.

### 3.3 Axonal/Dendritic Outgrowth and Sprouting

Interestingly, vesicular docking/fusion at the plasma membrane is responsible not only for the release of neurotransmitters, but also for surface expression of plasma membrane proteins and lipids. Therefore, exocytosis plays a fundamental role in axonal/dendritic outgrowth and sprouting because both processes involve major increases in the surface area of the plasma membrane. In addition, treatment with chronic antidepressant increases the expression of GAP-43 in the rat dentate gyrus (Chen et al., 2003). Because GAP-43 regulates growth of axons and modulates the formation of new connections, these findings suggest that chronic antidepressant treatment may have an effect on structural neuronal plasticity in the central nervous system. As mentioned above, ECT is a safe and the most effective treatment for severely depressed patients who are resistant to antidepressant medications. Interestingly, the common effects of antidepressants and ECT on connectivity and synaptic plasticity in the dentate gyrus are likely to relate to affective functions of depression (Stewart and Reid, 2000). Consistent with these findings are data demonstrating that chronic electroconvulsive seizure administration in animals induces sprouting of the granule cell mossy fiber pathway in the hippocampus (Vaidya et al., 1999).

## 4 Conclusion

In this article, we demonstrated that certain novel candidate molecular systems or pathways might underlie the mechanism of action of antidepressants. Defining the roles of these molecular systems in drug-induced neural plasticity is likely to transform the course of research on the biological basis of depression. Identification of such targets will advance future efforts in the quest to develop effective therapeutics that have a new mode of action in the brain. Such detailed knowledge will have profound effects on the diagnosis, prevention, and treatment of depression. In conclusion, in the era of functional genomics, novel biological approaches beyond the "monoamine hypothesis" are expected to evoke paradigm shifts in the future of depression research. Additional work will be necessary to test this hypothesis.

## References

Andriamampandry C, Muller C, Schmidt-Mutter C, Gobaille S, Spedding M, Aunis D, Maitre M (2002) Mss4 gene is up-regulated in rat brain after chronic treatment with antidepressant and down-regulated when rats are anhedonic. Mol Pharmacol 62: 1332-1338

Celano E, Tiraboschi E, Consogno E, D'Urso G, Mbakop MP, Gennarelli M, de Bartolomeis A, Racagni G, Popoli M. (2003) Selective regulation of presynaptic calcium/calmodulin-dependent kinase II by psychotropic drugs. Biol Psychiatry 53: 442-449

Chen B, Wang JF, Sun X, Young LT (2003) Regulation of GAP-43 expression by chronic desipramine treatment in rat cultured hippocampal cells, Biol Psychiatry 53: 530-537

D'Sa C, Duman RS (2002) Antidepressants and neuroplasticity. Bipolar Disord 4: 183-194

Duman RS (1998) Novel therapeutic approaches beyond the serotonin receptor. Biol Psychiatry 44: 324-335

Duman RS, Heninger GR, Nestler EJ (1997) A molecular and cellular theory of depression. Arch Gen Psychiatry 54: 597-606

Duman RS, Malberg J, Thome J (1999) Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 46: 1181-1191

Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH, Duman RS, Heninger GR, Nestler EJ (1998) Neurogenesis in the adult human hippocampus. Nat Med 4: 1313-1317

Golembiowska K, Dziubina A (2000) Effect of acute and chronic administration of citalopram on glutamate and aspartate release in the rat prefrontal cortex. Pol J Pharmacol 52: 441-448

Gould E, Tanapat P (1999) Stress and hippocampal neurogenesis. Biol Psychiatry 46: 1472-1479

Hellsten J, Wennstrom M, Mohapel P, Ekdahl CT, Bengzon J, Tingstrom A (2002) Electroconvulsive seizures increase hippocampal neurogenesis after chronic corticosterone treatment. Eur J Neurosci 16: 283-290

Honer WG, Falkai P, Bayer TA, Xie J, Hu L, Li HY, Arango V, Mann JJ, Dwork AJ, Trimble WS. (2002) Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. Cerebral Cortex 12: 349-356

Malberg JE, Eisch AJ, Nestler EJ and Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20: 9104-9110

Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 39: 569-578

Stewart CA, Reid IC (2000) Repeated ECS and fluoxetine administration have equivalent effects on hippocampal synaptic plasticity. Psychopharmacol 148: 217-223

Vaidya VA, Siuciak JA, Du F, Duman RS (1999) Hippocampal mossy fiber sprouting induced by chronic electroconvulsive seizures. Neurosci 89: 157-166

van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96: 13427-13431

Yamada M, Higuchi T (2002) Functional genomics and depression research. Eur Neuropsychopharmacol 12: 235-244

Yamada M, Takahashi K, Tsunoda M, Nishioka G, Kudo K, Ohata H, Kamijima K, Higuchi T, Momose K, Yamada M (2002) Differential expression of VAMP2/synaptobrevin-2 after antidepressant and electroconvulsive treatment in rat frontal cortex. Pharmacogenomics J 2: 377-382

Yamada M, Yamada M, Yamazaki S, Nara K, Kiuchi Y, Ozawa H, Yamada S,