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H. 知的財産権の出願・登録状況

1. 特許取得　なし
2. 実用新案登録　なし
3. その他　なし

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分担研究報告書

ゲノム医学を活用した統合失調症及び気分障害に対する個別化治療法の開発

分担研究者　山田光彦　国立精神・神経センター精神保健研究所　部長

研究要旨

嗅球部位を摘出し長期隔離飼育したラットは、正常ラットに比べ非常に激しい情動過多反応を示す。この情動過多反応の改善には既存の抗うつ薬の数週間の慢性投与を必要とする。したがって、我々は本モデルを薬物の長期投与可能な動物モデルとして捉え、慢性投与による抗うつ薬の作用機序を明らかにする有用なツールとして考えている。

そこで本研究では、嗅球摘出ラット及び同処置に抗うつ薬を慢性投与したラットの脳内遺伝子発現プロファイルを比較検討した。その結果、抗うつ薬投与により行動が改善したラットにおいて発現変化する遺伝子群を同定した。SSRI の代謝系、薬理作用を考慮したターゲット遺伝子の解析（CYP 系、セロトニン系、GABA 系、DA 系）に加え、我々が同定した抗うつ薬の作用機序に関連する候補遺伝子について遺伝子多型を検索することにより、統合失調症及び気分障害の患者の個別化医療の実現、画期的治療薬開発そして発症脆弱性遺伝子解明に迫れるものと考える。

A. 研究目的

現在、新規抗うつ薬の開発は神経伝達物質の薬理学に基づいて行われており、選択的セロトニン再取り込み阻害薬、セロトニン・ノルアドレナリン再取り込み阻害薬などの開発が進み一定の成果を上げている。しかしながら、これらの抗うつ薬も、シナプス間隙におけるモノアミン濃度を増加させることにより抗うつ作用を発揮するという「モノアミン仮説」に基づく抗うつ薬の限界を超えるものではない。こうした急性薬理作用が脳内において直ちに起こるのに対して、臨床場面では治療効果の発現に数週間かかることが経験的にわかっており、抗うつ効果の作用機序を考える上で、急性の薬理作用と慢性によって認められる神経化学的な脳内変化を区別して考える必要性がある。我々は、抗うつ薬の慢性投与によって認められる脳内変化こそが抗うつ薬の治癒機転であり、新規抗うつ薬創薬のターゲット分子システムであると考えている。

ラット脳内の嗅球を摘出し長期隔離飼育すると、正常ラットに比べ被刺激性、攻撃性が高まる等の情動過多反応を示す。また、高架式十字迷路試験においては、壁無し走行路への滞在時間の短縮を示す。これらの情動過多反応は、既存の抗うつ薬の数週間の慢性投与により改善される。したがって、我々は本モデルを薬物の長期投与可能なうつ病の動物モデルとして捉え、慢性投与による抗うつ薬の作用機序を明らかにする有用なツールとして考えている。そこで本研究では、嗅球摘出（OB）ラットを用い、イミプラミン慢性投与による行動変化に伴う脳内遺伝子発現プロファイルを比較検討することにより、抗うつ薬の作用機序に関与する遺伝子を探索することを目的とした。

B. 研究方法

<u>嗅球摘出ラットの作成</u>

実験には、Wister 系雄性ラット（日本チャールズリバー）7 週齢を用いた。ペントバルビタール（50 mg/ml/kg, 腹腔内）の麻酔下、左右嗅球部位（bregma から前方に 7mm、左右に 1.8mm）を

吸引・除去した後、頭皮を縫合し、2週間以上単独隔離飼育した。被刺激性の程度は Gomita ら(1983)の方法を基に作成した評価基準に従い採点を行い、各項目のスコアーの合計が 14 点以上の動物を、情動過多を示した動物として選択した。これらの動物を2群に分け、溶媒またはイミプラミン(10mg/kg)の慢性投与を行った。

GeneChip 解析による抗うつ薬作用機序に関与する遺伝子の探索

偽手術(sham)、嗅球摘出(OB)、OB ラットにイミプラミン投与の3群（各4匹）より total RNA を抽出し、Affymetrix 社 GeneChip expression analysis technical manual に従い増幅し、ビオチンラベルした cRNA を作成し、GeneChip rat genome 230 2.0 Array (Affymetrix) にハイブリダイズさせた。Streptavidin Phycoerythrin (SAPE)で染色し、蛍光画像をスキャナーで取り込み、その蛍光強度から各プローブの発現量を算出した。

すべての動物実験は、動物愛護上十分な配慮をし、NIH ガイドラインに準拠した動物実験プロトコールに基づき、施設内審査委員会の規定に則り倫理性と科学性に十分配慮して実施した。

C. 研究結果

嗅球摘出ラットにおける情動過多評価基準は Gomita ら(1983)の方法を基に作成した。A)鼻先に差し出した棒に対する反応、B)空気を吹きかけたときの反応、C)捕獲や取り扱いに対する抵抗性、D)尾を鉗子で挟んだときの反応、E)テスト中の鳴き声の5項目についてそれぞれ無反応から激しい反応の5段階（0から4点）のスコアーを採点し、合計スコアーで判定した。ラット嗅球部位を摘出し長期隔離飼育したラット(OB)は、偽手術を施したラット(sham)に比べ情動過多スコアーが有意な高値を示した。また、イミプラミン慢性投与により有意な低下を示し、情動過多反応が改善していることが示された。

そこでこれらの動物の前頭葉皮質より total RNA を抽出し、GeneChip rat genome 230 2.0 Array による遺伝子発現プロファイル解析を行った。発現が確認されたスポットは 18,509 遺伝子であった。OB と OB+イミプラミン群におけるそれぞれの遺伝子の平均の発現比に 1.5 倍以上の差があり、さらに統計処理（t-test 及び Benjamini test）により有意差が認められたものを「発現変化あり」と選別した。OB に比べて OB+イミプラミン投与で up-regulation していた遺伝子が 195 遺伝子、down-regulation していた遺伝子が 58 遺伝子であった。これらの遺伝子について機能別分類を行ったところ、細胞接着や細胞骨格、細胞周期、細胞内情報伝達系等に関与する遺伝子が含まれていた。

D. 考察

現在用いられている抗うつ薬のモノアミン再取り込み阻害作用等の薬理作用は比較的短時間に認められるものの、実際の臨床現場では抗うつ効果発現までに数週間の慢性投与が必要である。したがって、抗うつ効果の作用機序を考える上で、急性の薬理作用と慢性によって認められる神経化学的な脳内変化を区別して考える必要性がある。我々は、抗うつ薬の慢性投与によって認められる脳内変化こそが抗うつ薬の治癒機転であり、新規抗うつ薬創薬のターゲット分子システムであると考えている。

マウス絶望モデルは、逃避不可能な水槽中で強制的にマウスを水泳させ、泳いでいない状態（無動）を「うつ」と定義するもので、既存の抗うつ薬により無動時間の短縮が認められる。強制水泳試験法は、本モデルを用いて、薬物による無動時間の短縮を評価する方法で、薬物を簡便かつ迅速に評価できるだけでなく、臨床での薬効比と動物モデルでの効力比が一致するという特徴も有することから、従来から薬物の抗

うつ効果を評価するモデルとして数多くの報告がなされている。しかし、マウス絶望モデルにおける既存の抗うつ薬の無動時間短縮作用は単回投与でも認められることから、長期投与が必要なうつ病病態モデルとして十分であるとはいいきれない。

一方、嗅球を摘出したラットを長期隔離飼育する嗅球摘出モデルは、被刺激性・攻撃性が高じ、情動過多反応を示す。これらの情動過多反応の改善には既存の抗うつ薬の数週間の慢性投与を必要とする。したがって、我々は本モデルを薬物の長期投与可能な動物モデルとして捉え、慢性投与による抗うつ薬の作用機序を明らかにする有用なツールとして考えている。そこで本研究では、嗅球摘出（OB）ラットを用いイミプラミン慢性投与による行動変化に伴う脳内遺伝子発現プロファイルを比較検討することにより、抗うつ薬の作用機序に関与する遺伝子を探索した。これらの候補遺伝子の多型を、SSRI の代謝系、薬理作用を考慮したターゲット遺伝子の解析（CYP 系、セロトニン系、GABA 系、DA 系）に加え同定していくことにより、主任研究者が行う「統合失調症及び気分障害の患者の個別化医療の実現、画期的治療薬開発そして発症脆弱性遺伝子解明」に寄与するものと考えられた。

E. 結論

嗅球摘出ラットを用いて、抗うつ薬の作用機序に関与する考えられる候補遺伝子を選別、同定した。

F. 健康危険情報

特記すべきことなし。

G. 研究成果発表

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H. 知的財産権の出願・登録状況

1. 特許取得　なし
2. 実用新案　なし
3. その他　なし

Ⅲ. 研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

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

Misa Yamada[1, 2], Kou Takahashi[1, 2], Chika, Kurahashi[2], Mitsuhiko Yamada[1], and Kazuo Honda[2]

[1]Department of Psychogeriatrics, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8502, Japan
[2]Departments of Pharmacology, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8555, Japan

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 af-

ter 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.

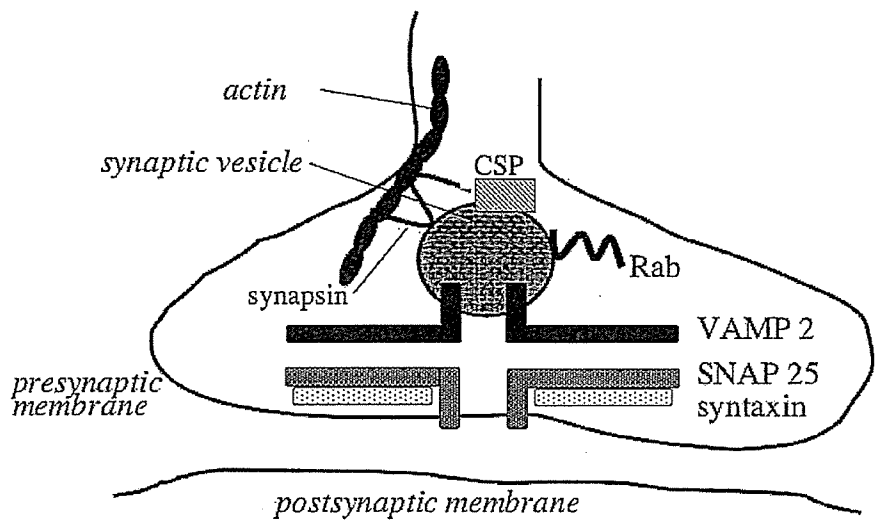

Fig. 1. Synaptic vesicle proteins identified as ADRG and related proteins. The expression of some of the ADRGs related to neurotransmiter release and located on synaptic vesicle including VAMP2/synaptobrevin, cysteine string protein (*CSP*), synapsin I, Rab-1A and Rab-3B were induced after chronic sertraline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

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Identification of Molecular Systems Responsible for the Therapeutic Effect of Antidepressant

Mitsuhiko Yamada[1, 2] and Misa Yamada[1, 3]

[1]Department of Psychogeriatrics, National Institute of Mental Health, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashimachi, Kodaira, Tokyo 187-8502, Japan
[2]Departments of Psychiatry, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8666, Japan
[3]Departments of Pharmacology, School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa, Tokyo 142-8555, Japan

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).

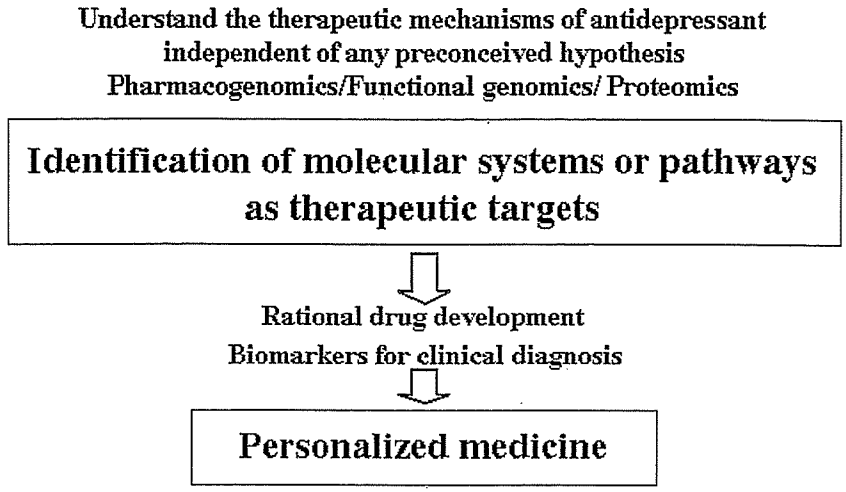

Fig. 1. Pharmacogenomics, Functional genomics and Proteomics are powerful tools that can be used to identify neuronal systems or pathways affected by antidepressants

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 elic-

ited 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