とにした。

B. 研究方法

S214A 変異タウコンストラクトの作成

野生型タウ（4リピート最長型）コンストラクトをもとに、Quick Change Kit (Stratagen)をもちいたPCR 法によって、S214A 変異を導入した。

野生型およびS214A 変異型タウの細胞への導入

COS-7 細胞を 5% FCS を含む D-MEM (Gibco BRL)にて培養し、Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA)を用いて、野生型および変異型タウをトランスフェクションした。トランスフェクションをおこなった 24 時間後および 48 時間後、この細胞を RIPA バッファー (50mM Tris-HCl、pH7.4、150mM NaCl、1mM EDTA、1mM EGTA、1% Triton X-100、1% Sodium deoxycholate、0.1% Sodium dodecylsulphate、Protease inhibitor cocktail (Sigma) 0.5μl/ml、10nM Okadaic acid）にて溶解した lysates を 4℃、40K x G にて遠心し、その supernatant を得た。この supernatant を電気泳動し、さらに抗タウ蛋白ポリクローナル抗体 H150（SantaCrus, CA, USA）をもちいたウエスタンブロットによってタウ蛋白の発現レベルを解析した。

パルスチェイス法によって分解過程の解析

トランスフェクションをおこなった 24 時間後、Met-free medium (GIBCO) にて 1 時間培養し、その 3 時間後に EXPRE35S 35S Protein Labeling Mix (Perkin Elmer Japan, Kanagawa, Japan)を用いた 40 μCi の [^{35}S]Met を含むメディウムにて培養し、PBS にて細胞を洗浄したのちに、0 時間後および 24 時間後の細胞を RIPA バッファーにて回収した。そして同様の処理にて supernatant を回収したのちに、抗タウ蛋白モノクローナル抗体 Tau-5 ((Calbiochem, San Diego, CA, USA)および Protein G–Sepharose (GE Healthcare, Uppsala, Sweden)をもちいた免疫沈降をおこない、ラベルされたタウ蛋白を回収した。このサンプルを用いて電気泳動を行い、オートラジオグラフィーを行って、タウ蛋白の分解過程を検討した。

細胞内局在の検討

タウ蛋白の細胞内における局在を検討するために、ProteoExtract™ Subcellular Proteome Extraction Kit (Calbiochem)を用いて、細胞分画処理をおこなった。トランスフェクションをおこなった 24 時間後、細胞を洗浄液にて洗浄し、buffer I を添加し 4°C で 10 分間溶出させ、500 x G にて 10 分間遠心し、supernatant を fraction I とした。同様の処理を buffer II および buffer III にておこない、それぞれ supernatant を fraction II および III とした。ここで、fractionI、II および III は、それぞれ、細胞質分画、膜/オルガネラ分画、核分画に対応している。これらを抗タウ蛋白ポリクローナル抗体 H150 を用いたウエスタンブロットによって解析した。

C. 研究結果

野生型タウまたはS214A 変異型タウを挿入したものをトランスフェクションし抗タウ蛋白ポリクローナル抗体 H150 をもちいたウエスタンブロットによってタウ蛋白の発現レベルを解析したところ、24 時間後および 48 時間後においてタウ蛋白のバンドの強度に変化はなく、差は認められなかった。そこで、より詳細に検討するためにパルスチェイス法によって分解過程を追跡した。抗タウ蛋白モノクローナル抗体 Tau-5 をもちいた免疫沈降をおこない、ラベルされたタウ蛋白を回収し、オートラジオグラフィーを行って定量したところ、タウ蛋白の残存率は 0 時間を 100%として、野生型タウでは 57.8%であるのに対し、S214A タウでは 25.8%と残存率は少なかった。このことは、Ser214 がリン酸化されうる状態にあるものよりもリン酸化されない状態にある方が分解は速いことを意味している。

次に、分解速度の差が、野生型タウおよび S214A タウのそれぞれの細胞内における局在の差に由来する可能性を考慮して、細胞分画法による検討を行った。キットにより分画された成分をウエスタンブロットにより解析した。強制発現されたタウ蛋白は、どちらも主に fraction I（細胞質分画）に存在し、fraction II（膜/オルガネラ分画）および III（核分画）には少量しか存在せず、野生型タウおよび変異型（S214A および P301L）タウ蛋白の局在の差は確認できなかった。

D. 考察と結論

我々はタウ蛋白と結合する蛋白として 14-3-3 蛋白に注目し、タウ蛋白と 14-3-3 蛋白に結合するタウ蛋白の部位は少なくとも 2 ケ所存在し、1 つは微小管結合ドメイン、もうひとつは Ser214 部位であることを今までに明らかにしていた(Sadik G, et al. J Neurochem 108:33-43,2009.)。特に後者はリン酸化することによって結合活性を有することから、この部位のリン酸化によって 14-3-3 蛋白との結合様式が変化することが推測された。14-3-3 蛋白は脳内可溶性蛋白の 1 %を占める発現量の多い蛋白であり、様々な蛋白と結合してターゲット蛋白の制御を行っている。そこで、今回は細

胞内におけるタウ蛋白の自己重合および分解過程に影響を与えているのではないかという可能性を考えて、検討を行った。結果としては、パルスチェイス法によって分解過程を追跡したところ、タウ蛋白の分解は野生型タウよりもS214A変異タウの方が速いというものであった。つまり、Ser214部位がリン酸化されうる状態にあるものよりもリン酸化されない状態にある方が分解は速いことを意味している。さらに、細胞分画法による検討を行ったところ、強制発現されたタウ蛋白は野生型タウも変異タウも主に細胞質に局在し、著明な差は認めらなかった。

今までの我々の検討からは、FTDP-17変異型タウ蛋白の分解は遅延しており、その原因はリン酸化が亢進したための分解耐性が亢進したのではないかというものであった(Yanagi K, et al. Psycogeriatrics 9:157-166, 2009.)。今回、Ser214がリン酸化されうる状態にあるものよりもリン酸化されない状態にある方が分解は速いという結果は、2つの可能性を示唆している。ひとつは、Ser214がリン酸化されると14-3-3蛋白との結合親和性が亢進し、細胞内での強い結合が予測されることから、2つの蛋白が複合体を形成することでプロテアーゼに対する分解耐性が亢進するという可能性である。例えば、NO synthaseはHSP90と複合体を形成することで、calpainによる分解に耐性を呈することが知られている(Avema m, et al. FASEB J 274:6116-6127,2007.)。このように、タウ蛋白と14-3-3との強固な複合体形成がプロテアーゼの切断部位へのアクセスを障害し、その結果として分解が遅延する可能性が考えられる。もうひとつは、リン酸化そのものが蛋白の分解耐性を亢進させる可能性である。どの部位のリン酸化が責任部位であるかは明らかではないが、タウ蛋白はリン酸化されることによってcalpainによる分解耐性を亢進させる(Litersky JM, et al. J Biol Chem. 267:1563-1568,1992.)。この場合、Ser214部位が責任部位であれば、細胞内での分解遅延の原因は蛋白そのものの性質であると考えることができる。どちらの可能性がより大きな意味を持つかは不明であるが、詳細をしるためにはさらにin vitroの検討が必要であると考えられる。

今回の検討から、タウ蛋白の分解処理を亢進させるためには、Ser214部位をリン酸化させないようにするということになる。しかし、このSer214部位のリン酸化は自己重合を阻害することも知られている。よって、治療論的にどちらの方向性が有効であるかは現時点では明瞭ではない。今後、この部位の擬似リン酸化アミノ酸としてSerをAspまたはGluに置換した変異タウコンストを作成し、さらに詳細な検討をおこなう必要がある。この点が明瞭となれば、アルツハイマー病を含むタウオパチーに対する、タウの分解制御および重合制御に関する治療法が開発される可能性が考えられる。

E. 健康危険情報

特記すべきことなし。

F. 研究発表

1. 論文発表

1) Kato K, Tanaka T, Sadik G, Baba M, Maruyama D, Yanagida K, Kodama T, Morihara T, Tagami S, Okochi M, Kudo T, Takeda M. Protein kinase C stabilizes X-linked inhibitor of apoptosis protein (XIAP) through phosphorylation at Ser87 to suppress apoptotic cell death. Psychogeriatrics 11:90-97,2011.
2) Takeda M, Tanaka T, Okochi M. New drugs for Alzheimer's disease in Japan. Psychiatry Clin Neurosci. (査読有) 65(5):399-404. 2011.
3) Kazui H, Yoshida T, Takaya M, Sugiyama H, Yamamoto D, Kito Y, Wada T, Nomura K, Yasuda Y, Yamamori H, Ohi K, Fukumoto M, Iike N, Iwase M, Morihara T, Tagami S, Shimosegawa E, Hatazawa J, Ikeda Y, Uchida E, Tanaka T, Kudo T, Hashimoto R, Takeda M. Different characteristics of cognitive impairment in elderly schizophrenia and Alzheimer's disease in the mild cognitive impairment stage. Dement Geriatr Cogn Dis Extra. 1(1):20-30,2011.
4) 田中稔久、武田雅俊 主観的認知機能障害(SCI)から軽度認知機能障害(MCI)へ 老年精神医学雑誌 22;Supple1; 45-52,2011.
5) 田中稔久、武田雅俊 神経変性とTDP43、プログラニュリン、タウ Cognition and Dementia 10; 10-16,2011.
6) 田中稔久、武田雅俊 Rivastigmineの薬理作用―Dual actionへの期待― 臨床精神薬理 14:1137-1142,2011.
7) 田中稔久、武田雅俊 リバスチグミンの基礎と臨床 a. 基礎 精神科 19(3):252-258,2011.

8）田中稔久、武田雅俊　特集：アルツハイマー病、開発中の治療薬—disease modifying therapy 最新医学66(9):2259-2276,2011.

9）武田雅俊、ラモン・カカベロス、工藤喬、田中稔久、田上真次、大河内正康、森原剛史、橋本亮太　アポリポ蛋白Eと精神神経疾患　精神神経学雑誌 113:773-781,2011

2. 学会発表

1) Tanaka T, Kato K, Yanagi K, Maruyama D, Takeda M　Involvment of protein kinase C in neuronal cell apoptosis by phosphorylation of X-linked inhibitor of apoptosis protein (XIAP) at Ser87. The 10th World Congress of Biological Psychiatry. May 29 – June 2, 2011, Prague, Czech Republic.

2) Morihara T, Hayashi N, Yokokoji M, Akatsu H, Saito Y, Suzuki T, Takamura A, Katayama T, Ito N, Nishitomi K, Kimura N, Kazui H, Yanagida K, Kato K, Yatsumi S, Kodama T, Tagami S, Okochi M, Tanaka T, Kudo T, Takeda M　Identification of a gene which controls Abeta accumulation using APP Tg mice with mixed genetic background: Splicing variant specific-effect of Kinesin Light Chain 1 (Klc1). Alzheimer's Association International Conference (AAIC2011) ,Parris, France, July 16-27, 2011.

3) Tanaka T, Sadik G, Yanagi K, Kato K, Takeda M　Accumulation and aggregation of tau protein in tauopathies. T he 3rd World Congress of Asian Psychiatry, Jul 31 – Aug 4, 2011, Melbourne, Australia.

4) Tanaka T, Yanagi K, Maruyama D, Takeda M Involvement of puromycin-sensitve aminopeptidase in metabolism of tau protein in cultured cells. The 15th Congress of the International Psychogeriatrics Association, Sept.6-9, 2011, Den Haag, Holland.

5) 田中稔久　神経化学カレッジ　「アルツハイマー病」　第 54 回日本神経化学会大会　2011,09,25 山代温泉（石川県）

6) 田中稔久　ランチョンセミナー　「タウの分子病態と神経変性」　第 54 回日本神経化学会大会 2011,09,28　山代温泉（石川県）

7) 林紀行、横小路美貴子、森原剛史、田上真次、大河内正康、田中稔久、工藤喬、武田雅俊　A beta 蓄積修飾遺伝子 KLC1 splicing variant の同定 (1) APP Tg マウスを用いた網羅的解析　第 30 回日本認知症学会　2011.11.11-13.　タワーホール船堀（東京都江戸川区）

8) 横小路美貴子、森原剛史、林紀行、木村展之、赤津裕康、高村明孝、片山泰一、斎藤有紀、鈴木利治、加藤希世子、辰巳真一、柳田寛太、児玉高志、田中稔久、武田雅俊　背景遺伝子が異なる APP Tg マウスの網羅的解析により同定された A beta 蓄積修飾遺伝子 KLC1 splicing variant の同定 (2) ヒトの脳、末梢リンパ球での発現解析　第 30 回日本認知症学会　2011.11.11-13.　タワーホール船堀（東京都江戸川区）

G. 知的財産権の出願・登録状況（予定を含む）

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

アルツハイマー病患者と軽度認知障害患者の精神行動障害

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研究要旨
アルツハイマー病（AD）治療薬は認知障害の改善、進行抑制とともに精神行動障害に対する効果を有することが望ましい。今年度、我々は大阪大学医学部附属病院神経科精神科神経心理専門外来を受診したアルツハイマー病（AD）患者50例と、ADの前段階と考えられる軽度認知障害（MCI）患者16例を対象にNeuropsychiatric Inventoryを用いて精神行動障害を評価した。その結果、ADとMCIの両群で多彩な精神行動障害を呈することが明らかになった。すなわち、ともに無為、興奮、不安が多く、多幸は認めなかった。精神行動障害を有する患者の率はAD患者の方がMCI患者よりも多かったが、精神行動障害を有する患者においては、症状の頻度と重症度は両群でほぼ同等であった。以上より、MCIにおいてもAD患者と同様の頻度と重症度の精神行動障害を呈しうることが明らかになった。現在、開発中のADに対する疾患修飾薬は、これまでの進行抑制薬よりもMCIのようなより早期の段階で投与開始されることが想定されている。本研究の結果より、このMCIの段階で新規薬を投与する際には精神行動障害に対する効果も評価すべきであると考えられた。

A. 研究目的

アルツハイマー病(Alzheimer's disease: AD)患者は認知障害だけでなく、精神行動障害も有する。従って、AD患者の治療薬は、認知障害だけでなく精神行動障害に対する有効性も判定しなければならない。今回我々は、AD患者とその前段階である軽度認知障害(Mild Cognitive Impairment: MCI)患者の精神行動障害の様態を明らかにした。

B. 研究方法

大阪大学医学部附属病院神経科精神科神経心理専門外来を受診し、以下のADとMCIの診断基準を満たす患者に対してNeuropsychiatric Inventory (NPI)で精神行動障害の評価を行った。ADの診断基準はNINCDS-ADRDAのprobable ADの診断基準を満たす患者とした。またMCIの診断基準はPetersenの基準で、物忘れを自覚、または他覚しているが、日常生活は自立している、Mini Mental State Examination (MMSE)＞23、Clinical Dementia Ratingが0. 5、Wechsler Memory Scale- Revised の論理的記憶検査で健常者の平均点から1. 5SD以上低いこととした。また頭部Magnetic Resonance Imagingや脳血流Single Photon Emission Computed Tomographyの結果も診断の参考とした。

(倫理面への配慮)
個人情報について厳重に管理し、データの解析は匿名化して行った。

C. 研究結果

1．ADの精神行動障害

対象となったADは50例であった。平均年齢は73.0±8.7歳、MMSEの合計点の平均は17.2±5.6点であった。NPIで評価した12の精神行動障害を有する患者の率は、多い順に、無為（44%）、興奮（42%）、不安（32%）、妄想（30%）、うつ(30%)、異常行動(19%)、易刺激性(17%)、食行動異常（12%）、睡眠障害（9%）、脱抑制（8%）、幻覚（4%）、多幸（0%）であった。それぞれの症状を有した患者についてNPIの頻度と重症度の平均を表1にまとめた。

2．MCIの精神行動障害

対象となったMCIは16例であった。平均年齢は74.1±9.5歳、MMSEの合計点の平均は25.9±1.6 点であった。NPIで評価した12の精神行動障害を有する患者の率は、多い順に、無為（63%）、興

奮（38%）、不安（25%）、うつ（19%）、妄想（13%）、睡眠障害（6%）、易刺激性（6%）、異常行動（0%）、食行動異常（0%）、脱抑制（0%）、幻覚（0%）、多幸（0%）であった。それぞれの症状を有した患者についてNPIの頻度と重症度の平均を表1にまとめた。

	AD		MCI	
	頻度	重症度	頻度	重症度
妄想	2.7	1.5	4.0	1.5
幻覚	1.5	1.0	−	−
興奮	2.7	1.7	2.7	1.0
うつ	2.2	1.1	3.0	1.3
不安	2.3	1.6	2.0	1.5
多幸	−	−	−	−
無為	3.7	1.6	3.9	1.1
脱抑制	1.4	1.4	−	−
易刺激性	2.5	1.5	2.0	1.0
異常行動	3.5	1.4	−	−
睡眠障害	3.6	1.0	3.0	1.0
食行動異常	3.8	1.3	−	−

D. 考察

AD群とMCI群で呈する精神行動障害には共通点があり、ともに無為、興奮、不安の順に多かった。また多幸は認められなかった。MCIでも様々な精神行動障害を呈することが明らかになったが、認める患者の率はADの方が多かった。精神行動障害を認める患者の、その症状の頻度については不安、易刺激性、睡眠障害についてはADで頻度が高かった。しかしMCIの方が頻度が多い症状もあり、それは妄想、うつ、無為であった。重症度についてはともに非常に強い症状はなく、ADの方が重症なのは、興奮、不安、無為、易刺激性であった。MCIの方が重度である症状は、うつであった。

今回の研究でMCIの段階でも多彩な精神行動障害を呈することが明らかになった。現在、ADに対する疾患修飾薬が開発されつつある。このような薬は進行抑制薬よりも、より早期のMCIの段階から投与を開始することが望ましいと考えられている。今回の結果より、疾患修飾薬の効果判定の際には、認知機能障害の改善、疾患の進行の抑制だけでなく、精神行動障害の改善もアウトカムに加えるべきと考える。

E. 結論

ADとMCI患者を対象にNPIを用いて精神行動障害を評価した。その結果、MCIの段階でも多彩な精神行動障害を呈することが明らかになった。この結果より、今後開発されるアルツハイマー病治療薬の薬効の判定には精神行動障害に対する効果も加えるべきと考えられた。

F. 研究発表

1．論文・書籍発表

・Kazui H, Yoshida T, et al. Different characteristics of cognitive impairment in elderly schizophrenia and Alzheimer's disease in the mild cognitive impairment stage. Dement Geriatr Cogn Disord Extra 1:20-30, 2011.

2．学会発表

・Nomura K, Kazui K, et al. Classification of delusions in Alzheimer's disease. International Psychogeriatric Association 15th International Congress, The Hague, The Netherlands, 6-9 September 2011.

・Nomura K, Kazui K, et al. Classification of delusions in Alzheimer's disease and the neural correlate. Korean Association for Geriatric Psychiatry annual meeting 2011 exchange program with Japan, Seoul, 2011.11.18.

G. 知的財産権の出願・登録状況（予定を含む。）

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

小胞体ストレスによる神経細胞内タウ凝集に関する研究

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研究要旨: 小胞体(ER)ストレスを負荷した神経細胞ではタウ蛋白量の上昇が観測される。この現象は、ERストレスによってタウに結合するE3ライゲースであるCHIP量が減少し、タウのユビキチン化が阻害され、プロテアソームにおけるタウの分解が抑制されることによることが予想された。本年度の検討により、ERストレス下では実際にタウに結合しているCHIPの量の低下が認められ、タウの分解が遅延することが示唆された。これらのことより、ERストレスを是正することで、神経細胞内のタウの凝集を抑止する可能性が考えられる。

キーワード：タウ、ERストレス、プロテアソーム、ユビキチン

A. 研究目的

小胞体(ER)ストレスはアルツハイマー病(AD)をはじめとする神経変性疾患の病理過程に関与するとされている。ADの神経細胞に観察されるタウの凝集はその産生と分解のバランスの破綻とも考えられる。本研究では、神経変性過程に広く関与するとされるERストレスのタウの産生および分解への関与について検討し、ERストレスを介したタウ凝集阻害法について考察する。

昨年度までの検討で、ERストレスによりタウのユビキチン化を担うE3ライゲースであるCHIPが減少することでタウのプロテアソームにおける分解が減少することが示唆されている。

B. 研究方法

HEK293細胞にタウのORFベクターをリポフェクションでトランスフェクションし、24時間静置する。培地中のグルコース除去を行った後、24時間後に細胞をRIPAバッファーで回収する。TAU-5抗体による免疫沈降を行い、抗CHIP抗体などによるウエスタンブロットを行った。

C. 研究結果

24時間グルコース除去を行う事により、eIF2αのリン酸化が生じ、ERストレスが付加されたことが確認された。このようなERストレス条件下で抗タウ抗体（Tau-5）によって免疫沈降されたCHIPは減少していた。すなわち、ERストレス下ではタウに結合するCHIPが減少しており、タウのユビキチン化およびプロテアソームにおける分解が遅延することが示唆された。

D. 考察

アミロイドカスケード仮説はADの病態仮説として広く受け入れられてきた。これを基盤としたDisease-Modyifing Therapyとして、アミロイドワクチンやγセクレターゼ阻害薬などの開発が現在精力的に行われている。しかし残念なことに、これらの多くの臨床治験結果は芳しいものではない。従って、アミロイド仮説に替わる病態仮説を基盤とした

AD の治療法開発が模索されるようになってきている。

AD 脳では神経原線維変化が観察されるように、タウ特にリン酸化タウが神経細胞内に凝集するという tauopathy の側面を持つ。近年、タウのノックアウトマウスではアミロイドが沈着しても神経細胞障害が起きない事が示され（Roberson, et al. Science, 2007）、tauopathy すなわちタウの凝集が治療ターゲットとして注目されるようになってきている。Tauopathy を呈する変性疾患は AD ばかりではなく、ピック病や進行性核上麻痺など多岐に及ぶが、ほとんどのケースが孤発性であり、タウ凝集のメカニズムについては不明である。

一方、AD をはじめとする変性性の認知症の病態過程に ER ストレスが関与しているという知見が、我々の検討（Katayama, -,Kudo, et al. Nature Cell Biology, 1999）（Katayama, -, Kudo, et al. J Biol Chem 2001）（Yasuda, Kudo, et al. Biochem Biophys Res Commun 2002）が端緒となり積み重ねられてきている。Tauopathy と ER ストレスに関しては、Hoozemans らのグループが、リン酸化タウの凝集に先立ち ER ストレスが生じていることを AD ばかりではなくピック病などの病理標本を用いて示唆している（Hoozemans, et al. Am J Pathol 2009）(Nijholt, et al. J Pathol 2012)。

本研究は ER ストレスが tauopathy をもたらすメカニズムについて検討を行った。グルコース除去、tunicamycin、thapsigargin、DTT で ER ストレスを神経細胞にかけると全てでタウ蛋白の上昇が観察された。

転写レベルでの検討として、タウの mRNA を検討したが、ER ストレスによる変化は認められなかった。

ER ストレス下では、ストレス反応として大部分の蛋白の翻訳が抑制されるが、ATF4 などの一部の蛋白はそれらの 5'UTR の特殊な構造により逆に翻訳が上昇するものもある (Harding, et.al., Mol. Cell, 2000)。そこで、タウの 5'UTR の有無により ER ストレス下でのタウ発現について比較検討した。24 時間あるいは 48 時間のグルコース除去による影響では、5'UTR の有無によるタウ蛋白発現の差は認められなかった。ATF4 を誘導する eIF2 α をリン酸化する PERK のノックアウト神経細胞においても、タウ蛋白の誘導は若干遅延するも認められた。これらのことより、ER ストレス下に見られるタウ蛋白の上昇は翻訳の影響ではないことが示唆された。

プロテアソームを阻害するとタウ蛋白は上昇してくることから、タウは主にユビキチン化されプロテアソームで分解されることが示された。タウをユビキチン化する E3 ライゲースとして GHIP が報告されている（Petrucelli, et al Hum Mol Gen 2004）。そこで、ER ストレス下における CHIP 変化について検討したところ、実際にタウに結合している CHIP が減少していた。すなわち、ER ストレスにより CHIP が減少し、タウのユビキチン化およびプロテアソームでの分解が遅延してタウ蛋白の上昇に繋がったと考えられた。

以上より、ER ストレスは主にタウの

分解過程に変化をもたらし、タウ蛋白の上昇を引き起こすと考えられ、ER ストレスに対する治療は tauopathy の治療になり得る事が示された。

E. 結論

ER ストレスによりタウに結合する CHIP は減少し、ユビキチン化およびプロテアソームにおける分解の遅延によりタウ蛋白が上昇する。

F. 健康危険情報

特になし。

G. 研究発表

1．論文発表

1. Kimura R, Morihara T, Kudo T, Kamino K, Takeda M Association between CAG repeat length in the PPP2R2B gene and Alzheimer disease in the Japanese population. Neurosci Let 2011; 487: 354-357.
2. Ishisaka M, Kudo T, Shimazawa M, Kakefuda K, Oyagi A, Hyakkoku K, Tsuruma K , Hara H Restraint-Induced Expression of Endoplasmic Reticulum Stress-Related Genes in the Mouse Brain Pharmacology & Pharmacy, 2011; 2(1): 10-16.
3. Furuichi T, Masuya H, Murakami T, Nishida K, Nishimura G, Suzuki T, Imaizumi K, Kudo T, Ohkawa K, Wakana S, Ikegawa S ENU-induced missense mutation in the C-propeptide coding region of Col2a1 creates a mouse of platyspondylic lethal skeletal dysplasia, Torrance type Mamm Genome 2011; 22: 318-328.
4. Kato K, Tanaka T, Sadik G, Baba M, Maruyama D, Yanagida K, Kodama T, Morihara T, Tagami S, Okochi M, Kudo T, Takeda M Protein kinase C stabilizes X-linked inhibitor of apoptosis protein (XIAP) through phosphorylation at Ser87 to suppress apoptotic cell death. Psychogeriatrics 2011; 11(2): 90-97.
5. Mitsuda T, Omi T, Tanimukai H, Sakagami Y, Tagami S, Okochi M, Kudo T, Takeda M Sigma-1Rs are upregulated via PERK/eIF2α/ATF4 pathway and execute protective function in ER stress. Biochem Biophys Res Commun. 2011; 415(3):519-525.

H. 知的財産権の出願・登録状況

特になし。

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kimura R, Morihara T, Kudo T, Kamino K, Takeda M	Association between CAG repeat length in the PPP2R2B gene and Alzheimer disease in the Japanese population.	Neurosci Let	487	354-357.	2011
Ishisaka M, Kudo T, Shimazawa M, Kakefuda K, Oyagi A, Hyakkoku K, Tsuruma K , Hara H	Restraint-Induced Expression of Endoplasmic Reticulum Stress-Related Genes in the Mouse Brain	Pharmacology & Pharmacy	2(1)	10-16	2011
Furuichi T, Masuya H, Murakami T, Nishida K, Nishimura G, Suzuki T, Imaizumi K, Kudo T, Ohkawa K, Wakana S, Ikegawa S	ENU-induced missense mutation in the C-propeptide coding region of Col2a1 creates a mouse of platyspondylic lethal skeletal dysplasia, Torrance type Mamm	Genome	22	318-328	2011
Kato K, Tanaka T, Sadik G, Baba M, Maruyama D, Yanagida K, Kodama T, Morihara T, Tagami S, Okochi M, Kudo T, Takeda M	Protein kinase C stabilizes X-linked inhibitor of apoptosis protein (XIAP) through phosphorylation at Ser87 to suppress apoptotic cell death.	Psychogeriatrics	11(2)	90-97	2011
Mitsuda T, Omi T, Tanimukai H, Sakagami Y, Tagami S, Okochi M, Kudo T, Takeda M	Sigma-1Rs are upregulated via PERK/eIF2α/ATF4 pathway and execute protective function in ER stress.	Biochem Biophys Res Commun.	415(3)	519-525	2011
Kazui H, Yoshida T, et al.	Different characteristics of cognitive impairment in elderly schizophrenia and Alzheimer's disease in the mild cognitive impairment stage.	Dement Geriatr Cogn Disord Extra	1	20-30	2011
Ono K, Li L, Takamura Y, Yoshiike Y, Zhu L, Han F, Mao X, Ikeda T, Takasaki J, Nishijo H, Takashima A, Teplow DB, Zagorski MG, Yamada M.	Phenolic Compounds Prevent Amyloid β-Protein Oligomerization and Synaptic Dysfunction by Site-specific Binding.	J Biol Chem.	287(18):14	631-43.	2012

Yoshiike Y, Yamashita S, Mizoroki T, Maeda S, Murayama M, Kimura T, Sahara N, Soeda Y, Takashima A.	Adaptive responses to alloxan-induced mild oxidative stress ameliorate certain tauopathy phenotypes.	Aging Cell.	11(1)	51-62.	2012
Mutsuga M, Chambers JK, Uchida K, Tei M, Makibuchi T, Mizorogi T, Takashima A, Nakayama H.	Binding of curcumin to senile plaques and cerebral amyloid angiopathy in the aged brain of various animals and to neurofibrillary tangles in Alzheimer's brain.	J Vet Med Sci.	74(1)	51-7	2012
Takasaki J, Ono K, Yoshiike Y, Hirohata M, Ikeda T, Morinaga A, Takashima A, Yamada M.	Vitamin A has anti-oligomerization effects on amyloid-β in vitro.	J Alzheimers Dis.	27(2)	271-80.	2011
Sotiropoulos I, Catania C, Pinto LG, Silva R, Pollerberg GE, Takashima A, Sousa N, Almeida OF.	Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits.	J Neurosci.	31(21)	7840-7.	2011
Kambe T, Motoi Y, Inoue R, Kojima N, Tada N, Kimura T, Sahara N, Yamashita S, Mizoroki T, Takashima A, Shimada K, Ishiguro K, Mizuma H, Onoe H, Mizuno Y, Hattori N.	Differential regional distribution of phosphorylated tau and synapse loss in the nucleus accumbens in tauopathy model mice.	Neurobiol Dis.	42(3)	404-14.	2011
Tsuji H, Nonaka T, Yamashita M, Suzukake M, Kametani F, Akiyama H, Mann DM, Tamaoka A and Hasegawa M	Epitope mapping of antibodies against TDP-43 and detection of protease-resistant fragments of pathological TDP-43 in amyotrophic lateral sclerosis and frontotemporal lobar degeneration.	Biochem Biophys Res Commun	417	116–121	2012
Iguchi Y, Katsuno M, Takagi S, Ishigaki S, Niwa JI, Hasegawa M, Tanaka F, Sobue G	Oxidative stress induced by glutathione depletion reproduces pathological modifications of TDP-43 linked to TDP-43 proteinopathies.	Neurobiol Dis.	45	862-70	2012

Foulds PG, Mitchell JD, Parker A, Turner R, Green G, Diggle P, Hasegawa M, Taylor M, Mann DM, and Allsop D	Phosphorylated alpha-synuclein can be detected in blood plasma and is potentially a useful biomarker for Parkinson's disease.	FASEB J	25	4127-37	2011
Foulds PG, Yokota O, Thurston A, Davidson Y, Ahmed Z, Holton J, Thompson JC, Akiyama H, Arai T, Hasegawa M, Gerhard A, Allsop and Mann DM	Post mortem cerebrospinal fluid α-synuclein levels are raised in multiple system atrophy and distinguish this from the other α-synucleinopathies, Parkinson's disease and Dementia with Lewy bodies.	Neurobiol Dis	45	188-95	2011
Hasegawa M, Nonaka T, Tsuji H, Tamaoka A, Yamashita M, Kametani F, Yoshida M, Arai T, Akiyama H	Molecular Dissection of TDP-43 Proteinopathies.	J Mol Neurosci	45	480-485	2011
Nonaka T and Hasegawa M	In vitro recapitulation of aberrant protein inclusions in neurodegenerative diseases, New cellular models of neurodegenerative diseases.	Commun & Integ Biol	4	501-502	2011
Meyerowitz J, Parker SJ, Vella LJ, Ng DCh, Price KA, Liddell JR, Caragounis A, Li QX, Masters CL, Nonaka T, Hasegawa M, Bogoyevitch MA, Kanninen KM, Crouch PJ, White AR	C-Jun N-terminal kinase controls TDP-43 accumulation in stress granules induced by oxidative stress.	Mol Neurodegener	6	57	2011

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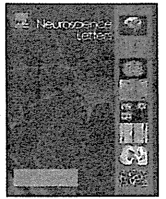

Association between CAG repeat length in the *PPP2R2B* gene and Alzheimer disease in the Japanese population

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ABSTRACT

We analyzed the association between *PPP2R2B* gene CAG repeat length and Alzheimer disease (AD) susceptibility in the Japanese population. Blood samples were collected from 218 late-onset AD patients and 86 controls. DNA fragments containing the target CAG repeat region were amplified using polymerase chain reaction (PCR). PCR products were sequenced using ABI PRISM 310 genetic analyzer. The mean CAG repeat length did not differ significantly between the control and AD groups. In contrast, the frequency of CAG repeats shorter than 15 was significantly higher in AD group, specifically in the AD with APOE4 subgroup, than in the control group. The results suggest that CAG repeat lengths in the *PPP2R2B* gene may be potential genetic markers for AD susceptibility in the Japanese population.

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Alzheimer disease (AD) is the most common cause of dementia in the elderly, and is characterized by progressive cognitive decline and cerebral atrophy. The primary pathological feature of AD is the presence of neurofibrillary tangles and senile plaques in the brain [26]. The presence of the ε4 allele of the apolipoprotein E (APOE) gene (*APOE4*) confers a heightened risk of late-onset AD in multiple genetic backgrounds [4]. Although trinucleotide repeats are common features of the human genome, the trinucleotide repeat number varies among individuals and the lengths of these repeats is associated with many genetic diseases, including Huntington disease (HD) and Dentatorubral-pallidoluysian atrophy (DRPLA) [25]. A majority of spinocerebellar ataxias (SCAs) are caused by the expansion of trinucleotide repeats. SCAs are a group of autosomal dominant progressive neurodegenerative disorders that are characterized by overlapping and variable phenotypes [20]. Spinocerebellar ataxia type 12 (SCA12) is caused by CAG repeat expansion in the non-coding region of the *PPP2R2B* gene [11]. Clinical symptoms of SCA12 include dementia, upper limb tremor, and extra pyramidal symptoms. Brain magnetic resonance images of the affected individuals revealed cerebral and cerebellar atrophy [11,23].

The *PPP2R2B* gene, which encodes a brain-specific regulatory B subunit of the serine/threonine protein phosphatase 2A (PP2A), is located on chromosome 5q31–33 and is widely expressed in brain neurons [21]. PP2A has been implicated in cell cycle and proliferation and development and regulation of multiple signal transduction pathways [30]. In addition, PP2A dephosphorylates the hyperphosphorylated tau protein [7]. It is suggested that PP2A-mediated dephosphorylation of tau is facilitated by the B regulatory subunit of PP2A [6]. Tau, an axonal microtubule-associated protein, promotes microtubule assembly and stabilization [17], and tau phosphorylation has been implicated, to varying degrees, in AD pathogenesis [12]. Because of the overlap between the SCA12 phenotype and certain aspects of AD, including the functional role of PP2A, it is important to determine the association between the *PPP2R2B* gene and AD. Recently, Chen et al. reported that the presence of short alleles of the CAG repeat in the *PPP2R2B* gene is associated with increased AD susceptibility in the Han Chinese [3]. However, the existence of such an association among other population group is uncertain. In the present study, we investigated the association between *PPP2R2B* gene CAG repeat lengths and AD susceptibility in the Japanese population.

Patients with late-onset AD were diagnosed with definite or probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke Alzheimer's Disease and Related Disorders Association [22]. The control group consisted of non-demented elderly subjects from the general population. After written informed consent was obtained, peripheral blood was collected from 218 late-onset AD patients (mean age: 79.0 years; women: 65.6%) and 86 control subjects (mean age: 74.7 years; women: 52.3%). The protocol for specimen collection was approved by the Genome Ethical Committee of Osaka University Graduate School of Medicine.

DNA was extracted from peripheral blood nuclear cells using the phenol–chloroform method or the QIAamp DNA Blood Kit (Qiagen). CAG repeats in the *PPP2R2B* gene were identified

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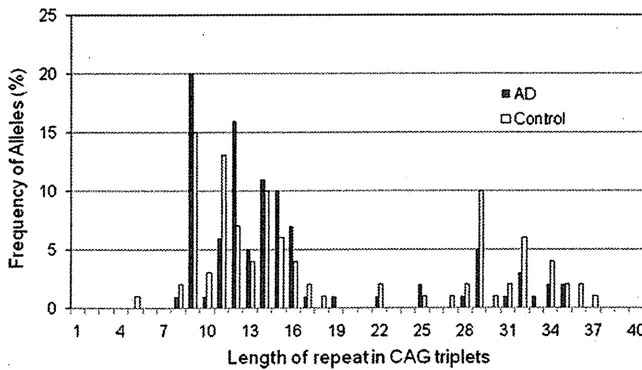

Fig. 1. Distribution of allele frequencies against the CAG repeat numbers in the *PPP2R2B* gene of control subjects and AD patients.

by polymerase chain reaction (PCR) amplification using 6FAM dye-labeled forward (5′-TGCTGGGAAAGAGTCGTG-3′) and reverse (5′-GCCCGCGCACTCACCCTC-3′) primers. The PCR was performed with 36 cycles consisting of two cycles of 30 s at 95 °C and 30 s at 70 °C, two cycles of 30 s at 95 °C and 30 s at 65 °C, two cycles of 30 s at 95 °C and 30 s at 60 °C, and 30 cycles of 30 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C preceded by 10 min at 95 °C and followed by 10 min at 72 °C. PCR products were electrophoresed in a capillary in an automated ABI PRISM 310 genetic analyzer (Applied Biosystems). Analysis was performed with GenScan analysis software (Applied Biosystems) [11]. The *APOE* genotype was determined using a PCR-RFLP method [15].

Statistical analysis was performed using JMP (version 7.0, SAS Institute, Cary, NC). The 2-sided Mann–Whitney's *U*-test was used to evaluate the difference in CAG repeat distribution between the AD and control groups. The difference in the CAG repeat allele frequencies between the groups was further tested by the Chi-square test. Each value represents mean (standard error). A *p*-value of <0.05 was considered statistically significant.

The frequency distribution of CAG repeat alleles in the *PPP2R2B* genes was analyzed in 218 LOAD patients and 86 controls. In Fig. 1, the CAG repeat number (*X*-axis) is plotted against the frequency of distributions (%) (*Y*-axis). The repeat range was 5–37 and 8–35 in the control and AD groups, respectively. Pathological expansion of CAG repeats was not detected in the AD and control groups. The most common lengths were 9 (15.3%) triplets in the control group. Similarly, in the AD group, the most common lengths were 9 (20.0%) triplets. The mean CAG repeat lengths in the AD and control groups (14.2 and 16.6, respectively) were not statistically different ($p = 0.158$). In addition, when we divided the AD group into APOE4 and non-APOE4 subgroups, we found that the mean CAG repeat lengths of both subgroups (13.9 and 14.5, respectively) were not significantly different from that of the control group (Table 1).

Table 2
Short (≤15) and long (15>) alleles: CAG repeat number in *PPP2R2B*; the short and long allele repeat numbers in the AD and control groups were compared.

Group	Allele number			p value	OR
	Total	Short (≤15)	Long (>15)		
Control	172	110 (64%)	62 (36%)		
Control with APOE4	24	16 (67%)	8 (33%)	0.267	
Control without APOE4	148	94 (64%)	54 (36%)	0.022*	1.58
AD	436	320 (73%)	116 (27%)	0.021*	1.55
AD with APOE4	212	163 (77%)	49 (23%)	0.005*	1.87
AD without APOE4	224	157 (70%)	67 (30%)	0.197	

Differences in the allele repeat numbers in the AD and control groups were determined using Chi-square test.
* $p < 0.05$, statistically significant.
OR, odds ratio.

Because the mean CAG repeat length among all subjects was 15, we dichotomized the alleles into short (≤15) and long (15>) categories. Statistical analysis revealed that the frequency of CAG repeats shorter than 15 was significantly higher in the AD group than in the control group ($p = 0.021$, odds ratio = 1.55) (Table 2). Compared to the controls, the AD subgroups, APOE4 and non-APOE4, each had a significantly higher frequency of CAG repeats shorter than 15 ($p = 0.005$, odds ratio = 1.87). However, there was no significant difference in the allele frequency distribution between the non-APOE4 AD group and the control group ($p = 0.197$) (Table 2). Additionally, a comparison of the allele frequency distributions of the control subgroups, APOE4 and non-APOE4 with that of the AD revealed that the frequency of CAG repeats shorter than 15 was significantly higher in the AD groups than in the control without APOE4 groups ($p = 0.022$, odds ratio = 1.58) (Table 2).

SCA12 is a relatively rare late-onset neurodegenerative disorder characterized by diffuse cerebral and cerebellar atrophy [11]. The phenotype typically involves action tremor of upper extremities and various symptoms, including dementia. SCA12 is caused by CAG repeat expansion in the non-coding region of the *PPP2R2B* gene [10,11]. Pathogenic CAG repeat expansions have been detected in SCA12 patients in the range of 55–69 to 66–78, but normal individuals from different ethnic populations have exhibited ranges from 7–28 to 9–45 [2,3,5,11,27–29]. A correlation between the SCA12 phenotype and certain aspects of AD has been suggested. However, the lone study that analyzed the association between CAG repeat expansions in the *PPP2R2B* gene and AD susceptibility reported that the frequency of the Han Chinese individuals carrying the short 5-, 6-, and 7-triplet alleles was notably higher in AD patients [3].

In the present study, we investigated the length of *PPP2R2B* gene CAG repeats in AD patients and control subjects in the Japanese population. The mean CAG repeat lengths in the AD and control groups were not statistically different. In contrast, we found that the frequency of CAG repeats shorter than 15 was significantly higher in the AD group, specifically the AD with APOE4 subgroup

Table 1
Comparison of CAG repeat numbers in control subjects and AD patients.

Group	Control			AD		
	Total	APOE4 (+)	APOE4 (−)	Total	APOE4 (+)	APOE4 (−)
Number	86	12	74	218	106	112
Allele range	5–37	9–34	5–37	8–35	8–35	8–35
Allele with maximum frequency						
Allele	9	9	9	9	9	9
Frequency (%)	15.3	14.2	16.7	20.0	20.1	17.5
Mean (SE)	16.6 (0.8)	14.4 (1.8)	16.9 (0.8)	14.2 (0.5)	13.9 (0.6)	14.5 (0.7)
p value		0.942	0.114	0.158	0.110	0.362

The differences between the CAG repeat numbers in the control and AD groups were assayed using Mann–Whitney's *U*-test.
SE: standard error of the mean.

than in the control group (Table 2). Our results suggested that AD is associated with a lower number of CAG repeats in the *PPP2R2B* gene. This was similar to the findings of a previous report by Chen et al. [3]. However, in our AD patients, we did not find short 5–7 triplet alleles which detected in AD patients in the Han Chinese population. This discrepancy may reflect a genetic differentiation between the Han Chinese and Japanese populations.

The presence of the ε4 allele of *APOE* gene confers a heightened risk of late-onset AD [4]. As compared to individuals without the ε4 alleles, the risk for AD is 2- to 3-fold and about 12-fold higher in individuals carrying one and two ε4 alleles, respectively [1,14,24]. Though several studies have attempted to elucidate the mechanism for this increased risk, how APOE4 influences AD progression has yet to be proven. In particular, we found that the frequency of short CAG repeats (≤15) was higher in the AD with APOE4 group than in the control group. Therefore, it is likely that a short number of CAG repeats of *PPP2R2B* gene play an important role for the progression of late-onset AD with APOE4.

PP2A is composed of three subunits: a catalytic subunit (C), a scaffolding subunit (A), and a regulatory subunit (B). Assembly of the complex with the regulatory B subunit is required for the specificity and regulation of PP2A [31]. In addition, PP2A is the major tau phosphatase that dephosphorylates tau at multiple sites, and its activity is decreased by 30% in the frontal or temporal cortex of AD patients compared to controls [8,18]. This down-regulation of PP2A activity in AD brains is thought to be partially responsible for abnormal tau phosphorylation. Therefore, differences in the CAG repeat lengths in the *PPP2R2B* gene may regulate PP2A activity, leading to AD progression. Through a reporter assay, the short 5–7 triplet alleles were shown to be associated with decreased PPP2R2B promoter activities [3]. However, it has not yet been demonstrated that the short CAG repeat lengths in the PPP2R2B affect PP2A function directly.

APOE plays an important role in the distribution and metabolism of cholesterol in the human body [19]. APOE4 has also been associated with tau hyperphosphorylation in several animal models [9]. In particular, high cholesterol such as in Niemann-Pick C disease might be involved in decreasing membrane fluidity [16]. Therefore, it was recently supposed that signal transduction through the interaction of APOE4 with the neuronal cell membrane might involve AD progression through various kinases and phosphatases [13].

In conclusion, our results suggest that CAG repeat lengths in the *PPP2R2B* gene may be potential genetic markers for AD susceptibility in the Japanese population. Further investigations are required to confirm the role of the *PPP2R2B* gene in AD using a larger sample size and a different population group.

Conflicts of interest

None of the authors has any conflicts of interest.

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References

[1] L. Bertram, M.B. McQueen, K. Mullin, D. Blacker, R.E. Tanzi, Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database, Nat. Genet. 39 (2007) 17–23.

[2] A. Brusco, C. Cagnoli, A. Franco, E. Dragone, A. Nardacchione, E. Grosso, P. Mortara, R. Mutani, N. Migone, L. Orsi, Analysis of SCA8 and SCA12 loci in 134 Italian ataxic patients negative for SCA1-3, 6 and 7 CAG expansions, J. Neurol. 249 (2002) 923–929.

[3] C.M. Chen, Y.T. Hou, J.Y. Liu, Y.R. Wu, C.H. Lin, H.C. Fung, W.C. Hsu, Y. Hsu, S.H. Lee, H.M. Hsieh-Li, M.T. Su, S.T. Chen, H.Y. Lane, G.J. Lee-Chen, PPP2R2B CAG repeat length in the Han Chinese in Taiwan: association analyses in neurological and psychiatric disorders and potential functional implications, Am. J. Med. Genet. B: Neuropsychiatr. Genet. 150B (2009) 124–129.

[4] E.H. Corder, A.M. Saunders, W.J. Strittmatter, D.E. Schmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines, M.A. Pericak-Vance, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, Science 261 (1993) 921–923.

[5] H. Fujigasaki, I.C. Verma, A. Camuzat, R.L. Margolis, C. Zander, A.S. Lebre, L. Jamot, R. Saxena, I. Anand, S.E. Holmes, C.A. Ross, A. Durr, A. Brice, SCA12 is a rare locus for autosomal dominant cerebellar ataxia: a study of an Indian family, Ann. Neurol. 49 (2001) 117–121.

[6] C.X. Gong, I. Grundke-Iqbal, K. Iqbal, Dephosphorylation of Alzheimer's disease abnormally phosphorylated tau by protein phosphatase-2A, Neuroscience 61 (1994) 765–772.

[7] C.X. Gong, T. Lidsky, J. Wegiel, L. Zuck, I. Grundke-Iqbal, K. Iqbal, Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain. Implications for neurofibrillary degeneration in Alzheimer's disease, J. Biol. Chem. 275 (2000) 5535–5544.

[8] C.X. Gong, S. Shaikh, J.Z. Wang, T. Zaidi, I. Grundke-Iqbal, K. Iqbal, Phosphatase activity toward abnormally phosphorylated tau: decrease in Alzheimer disease brain, J. Neurochem. 65 (1995) 732–738.

[9] F.M. Harris, W.J. Brecht, Q. Xu, R.W. Mahley, Y. Huang, Increased tau phosphorylation in apolipoprotein E4 transgenic mice is associated with activation of extracellular signal-regulated kinase: modulation by zinc, J. Biol. Chem. 279 (2004) 44795–44801.

[10] S.E. Holmes, E.O. Hearn, C.A. Ross, R.L. Margolis, SCA12: an unusual mutation leads to an unusual spinocerebellar ataxia, Brain Res. Bull. 56 (2001) 397–403.

[11] S.E. Holmes, E.E. O'Hearn, M.G. McInnis, D.A. Gorelick-Feldman, J.J. Kleiderlein, C. Callahan, N.G. Kwak, R.G. Ingersoll-Ashworth, M. Sherr, A.J. Sumner, A.H. Sharp, U. Ananth, W.K. Seltzer, M.A. Boss, A.M. Vieria-Saecker, J.T. Epplen, O. Riess, C.A. Ross, R.L. Margolis, Expansion of a novel CAG trinucleotide repeat in the 5′ region of PPP2R2B is associated with SCA12, Nat. Genet. 23 (1999) 391–392.

[12] K. Iqbal, C. Alonso Adel, S. Chen, M.O. Chohan, E. El-Akkad, C.X. Gong, S. Khatoon, B. Li, F. Liu, A. Rahman, H. Tanimukai, I. Grundke-Iqbal, Tau pathology in Alzheimer disease and other tauopathies, Biochim. Biophys. Acta 1739 (2005) 198–210.

[13] K. Iqbal, F. Liu, C.X. Gong, C. Alonso Adel, I. Grundke-Iqbal, Mechanisms of tau-induced neurodegeneration, Acta Neuropathol. 118 (2009) 53–69.

[14] J. Kim, J.M. Basak, D.M. Holtzman, The role of apolipoprotein E in Alzheimer's disease, Neuron 63 (2009) 287–303.

[15] R. Kimura, K. Kamino, M. Yamamoto, A. Nuripa, T. Kida, H. Kazui, R. Hashimoto, T. Tanaka, T. Kudo, H. Yamagata, Y. Tabara, T. Miki, H. Akatsu, K. Kosaka, E. Funakoshi, K. Nishitomi, G. Sakaguchi, A. Kato, H. Hattori, T. Uema, M. Takeda, The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between beta-amyloid production and tau phosphorylation in Alzheimer disease, Hum. Mol. Genet. 16 (2007) 15–23.

[16] Z Korade, A.K. Kenworthy, Lipid rafts, cholesterol, and the brain, Neuropharmacology 55 (2008) 1265–1273.

[17] V.M. Lee, M. Goedert, J.Q. Trojanowski, Neurodegenerative tauopathies, Annu. Rev. Neurosci. 24 (2001) 1121–1159.

[18] F. Liu, I. Grundke-Iqbal, K. Iqbal, C.X. Gong, Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation, Eur. J. Neurosci. 22 (2005) 1942–1950.

[19] R.W. Mahley, B.P. Nathan, R.E. Pitas, E. Apolipoprotein, Structure, function, and possible roles in Alzheimer's disease, Ann. N. Y. Acad. Sci. 777 (1996) 139–145.

[20] M.U Manto, The wide spectrum of spinocerebellar ataxias (SCAs), Cerebellum 4 (2005) 2–6.

[21] R.E. Mayer, P. Hendrix, P. Cron, R. Matthies, S.R. Stone, J. Goris, W. Merlevede, J. Hofsteenge, B.A. Hemmings, Structure of the 55-kDa regulatory subunit of protein phosphatase 2A: evidence for a neuronal-specific isoform, Biochemistry 30 (1991) 3589–3597.

[22] G. McKhann, D. Drachman, M. Folstein, R. Katzman, D. Price, E.M. Stadlan, Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease, Neurology 34 (1984) 939–944.

[23] E. O'Hearn, S.E. Holmes, P.C. Calvert, C.A. Ross, R.L. Margolis, SCA-12: tremor with cerebellar and cortical atrophy is associated with a CAG repeat expansion, Neurology 56 (2001) 299–303.

[24] A.D. Roses, Apolipoprotein E alleles as risk factors in Alzheimer's disease, Annu. Rev. Med. 47 (1996) 387–400.

[25] C.A. Ross, Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders, Neuron 35 (2002) 819–822.

[26] D.J. Selkoe, Alzheimer's disease is a synaptic failure, Science 298 (2002) 789–791.

[27] A.K. Srivastava, S. Choudhry, M.S. Gopinath, S. Roy, M. Tripathi, S.K. Brahmachari, S. Jain, Molecular and clinical correlation in five Indian families with spinocerebellar ataxia 12, Ann. Neurol. 50 (2001) 796–800.

[28] A. Sulek, D. Hoffman-Zacharska, M. Bednarska-Makaruk, W. Szirkowiec, J. Zaremba, Polymorphism of trinucleotide repeats in non-translated regions of SCA8 and SCA12 genes: allele distribution in a Polish control group, J. Appl. Genet. 45 (2004) 101–105.
[29] H.F. Tsai, C.S. Liu, T.M. Leu, F.C. Wen, S.J. Lin, C.C. Liu, D.K. Yang, C. Li, M. Hsieh, Analysis of trinucleotide repeats in different SCA loci in spinocerebellar ataxia patients and in normal population of Taiwan, Acta Neurol. Scand. 109 (2004) 355–360.
[30] D.M. Virshup, Protein phosphatase 2A: a panoply of enzymes, Curr. Opin. Cell. Biol. 12 (2000) 180–185.
[31] Y. Xu, Y. Chen, P. Zhang, P.D. Jeffrey, Y. Shi, Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation, Mol. Cell 31 (2008) 873–885.

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Scientific Research

Restraint-Induced Expression of Endoplasmic Reticulum Stress-Related Genes in the Mouse Brain

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ABSTRACT

Depression is a significant public health concern but its pathology remains unclear. Previously, increases in an endoplasmic reticulum (ER) stress-related protein were reported in the temporal cortex of subjects with major depressive disorder who had died by suicide. This finding suggests an association between depression and ER stress. The present study was designed to investigate whether acute stress could affect the ER stress response. Mice were immobilized for a period of 6 hr and then expression of ER stress response-related genes was measured by real-time PCR. We also used enzyme-linked immunosorbent assay for concomitant measurement of the plasma corticosterone levels in the mice. The effect of corticosterone on ER stress proteins was further investigated by treating mice with corticosterone for 2 weeks and then measuring ER protein expression by Western blotting. After a 6 hr restraint stress, mRNA levels of ER stress-related genes, such as the 78-kilodalton glucose regulated protein (GRP78), the 94-kilodalton glucose regulated protein (GRP94), and calreticulin, were increased in the cortex, hippocampus, and striatum of mouse brain. Blood plasma corticosterone level was also increased. In the corticosterone-treated mouse model, the expression of GRP78 and GRP94 was significantly increased in the hippocampus. These results suggest that acute stress may affect ER function and that ER stress may be involved in the pathogenesis of restraint stress, including the development of depression.

Keywords: *Corticosterone, Depression, Endoplasmic Reticulum Stress, Restraint Stress*

1. Introduction

Major depression, along with bipolar disorder, has become a common psychiatric disorder in modern society. About 1% of the population is estimated to be affected by major depression one or more times during their lifetime [1]. Even though extensive studies have led to a variety of hypotheses regarding the molecular mechanism underlying depression, the pathogenesis of this disorder remains to be fully elucidated.

The endoplasmic reticulum (ER) is the cell organelle where secretory and membrane proteins are synthesized and folded. It also functions as a Ca^{2+} store and resource of calcium signals. The disturbance of ER functions through events such as disruption of Ca^{2+} homeostasis, inhibition of protein glycosylation or disulfide bond formation, hypoxia and viral or bacterial infection, can result in the accumulation of unfolded or misfolded proteins and may trigger stress responses in the cell (ER stress). To overcome ER stress, an unfolded protein response (UPR) is invoked by the activation of several signaling pathways; this UPR promotes an adaptive response to ER stress and reestablishes homeostasis in the ER [2,3]. Molecular chaperones such as the 78-kilodalton glucose regulated protein (GRP78) and the 94-kilodalton glucose regulated protein are induced and promote correct protein folding. If the damage is too severe to repair, C/EBP-homologous protein (CHOP) and other factors are activated and induce cell apoptosis [4]. On the other hand, if misfolded protein aggregates into insoluble higher-order structures, it can give rise to various diseases. For example, rhodopsin misfolding causes autosomal dominant retinitis pigmentosa [5], while the accumulation of amyloid β-peptide is associated with Alzheimer's disease [6].

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Some reports have also suggested a relationship between mental disorder and ER stress. In bipolar disorder patients, DNA microarray analysis of cell derived from twins discordant with respect to the disease revealed a down-regulated expression of genes related to ER stress responses such as x-box binding protein 1 (XBP1) and GRP78 [7]. In schizophrenia patients, a similar abnormality of these genes was found [8]. In addition, mood-stabilizing drugs such as valproate and lithium have been reported to increase the expression of GRP78, GRP94, and calreticulin [9]. Similarly, olanzapine, one of the second-generation "atypical" anti-psychotic drugs, appears to potentiates neuronal survival and neural stem cell differentiation by regulation of ER stress response proteins [10].

A recent study reported that significantly increased levels of GRP78, GRP94, and calreticulin were found in the temporal cortex of subjects with major depressive disorder who had died by suicide compared with control subjects who had died of other causes [11]. In addition, hippocampal atrophy [12] and reduction of glial density in the subgenual prefrontal cortex [13] were found in patients with major depression. Stress, a risk factor for depression, has been shown to induce atrophy of the apical dendrites of the hippocampal neurons [14], and to promote neuronal apoptosis in the cerebral cortex [15] in animal depression models. These findings suggest that a stressful situation, which may increase the risk for suicide, serves as an ER stressor. To clarify the relationship between exogenous stress and ER stress, in the present study, we investigated the expression of ER stress-related genes after restraint stress. We also focused on the elevation of corticosterone in the plasma and used a corticosterone-treated depression model to clarify the relationship between chronic corticosterone elevation and ER stress.

2. Materials and Methods

2.1. Animals

Male 9-week-old ddY mice and male 6-week-old ICR mice (Japan SLC, Hamamatsu, Japan) were used for all experiments. Mice were housed at 24 ± 2°C under a 12 hr light-dark cycle (lights on from 8:00 to 20:00) and had ad libitum access to food and water when not under restraint. Animals were acclimatized to laboratory conditions before the experiment. All procedures relating to animal care and treatment conformed to the animal care guidelines of the Animal Experiment Committee of Gifu Pharmaceutical University. All efforts were made to minimize both suffering and the number of animal used.

2.2. Restraint Stress

Male 9-week-old ddY mice (Japan SLC) weighing 30-40 g were used for real-time PCR studies. Mice were placed into 50-mL perforated plastic tubes, which prevented them from turning in any direction. Each mouse was maintained in the tube for 6 hr without any access to food or water.

2.3. Sampling

After this restraint stress, a blood sample was collected from the tail and the mouse was decapitated. The brain was quickly removed from the skull, briefly washed in ice-cold saline, and laid on a cooled (4°C) metal plate. The brain was rapidly dissected to separate the hippocampus, striatum, and cortex and stored at -80°C until use.

2.4. RNA Isolation

Total RNA was isolated from frozen brain using High Pure RNA Isolation Kit (Roche, Tokyo, Japan). RNA concentrations were determined spectrophotometrically at 260 nm. First-standed cDNA was synthesized in a 20-μl reaction volum using a random primer (Takara, Shiga, Japan) and Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA, USA).

2.5. Reai-Time PCR

Real-time PCR (TaqMan; Applied Biosystems, Foster City, CA, USA) was performed as described previously [16]. Single-standard cDNA was synthesized from total RNA using a high capacity cDNA archive kit (Applied Biosystems). Quantitative real-time PCR was performed using a sequence detection system (ABI PRISM 7900HT; Applied Biosystems) with a PCR master mix (TaqMan Universal PCR Master Mix; Applied Biosystems), according to the manufacturer's protocol. A gene expression product (Assays-on-Demand Gene Expression Product; Applied Biosystems) was used for measurements of mRNA expression by real-time PCR. The primers used for amplification were as follows: GRP78: 5'-GTTTG CTGAGGAAGACAAAAAGCTC-3' and 5'-CACTTCC ATAGAGTTTGCTGATAATTG-3'; CHOP: 5'-GGAG CTGGAAGCCTGGTATGAGG-3' and 5'-TCCCTGGT CAGGCGCTCGATTTCC-3'; GRP94: 5'-CTCACCATT TGGATCCTGTGTG-3' and 5'-CACATGACAAGATT TTACATCAAGA-3'; calreticulin: 5'-GCCAAGGACG AGCTGTAGAGAG-3' and 5'-GGTGAGGGCTGAAG GAGAATC-3'; ERdj4: 5'-TCTAGAATGGCTACTCCC CAGTCAATTTTC-3' and 5'-TCTAGACTACTGTCCT GAACAGTCAGTG-3'; EDEM: 5'-TGGGTTGGAAAG CAGAGTGGC-3' and 5'-TCCATTCCTACATGGAGG TAG-3'; p58IPK 5'-GAGGTTTGTGTTTGGGATGCAG-3' and 5'-GCTCTTCAGCTGACTCAATCAG-3'; ASNS: 5'-AGGTTGATGATGCAATGATGG-3' and 5'-TCCC CTATCTACCCACAGTCC-3'; β-actin: 5'-TCCTCCCT

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GGAGAAGAGCTAC-3' and 5'-TCCTGCTTGCTGAT CCACAT-3' The thermal cycler conditions were as follows: 2 min at 50°C and then 10 min at 95°C, followed by two-step PCR for 50 cycles consisting of 95°C for 15s followed by 60°C for 1 min. For each PCR measurement, we checked the slope value, R^2 value, and linear range of a standard curve of serial dilutions. All reactions were performed in duplicate. The results were expressed relative to a β-actin internal control.

2.6. Measurement of Plasma Corticosterone

Plasma was obtained as described previously [17] and the concentration of corticosterone was determined *via* a corticosterone EIA kit (Assay Designs, Inc., Ann Arbor, MI, USA) according to the manufacturer's protocol.

2.7. Chronic Corticosterone Treatment

Male 6-week-old ICR mice (Japan SLC) weighing 20-25 g were used for chronic oral corticosterone exposure as described in a previous report [18]. Briefly, corticosterone (25 µg/mL free base; 4-pregnen-11β 21-DIOL-3 20-DIONE 21-hemisuccinate; Steraloids, Inc., RI, USA) was add to tap water and the pH was brought to 12-13 with 10 N NaOH (Kishidai Chemical, Osaka, Japan), followed by stirring at 4°C until dissolved (3 to 7 hr). Following dissolution, the pH was brought to 7.0-7.4 with 10 N HCl (Wako, Osaka, Japan). Group-housed ICR mice were presented with this corticosterone solution in place of normal drinking water for 14 days, resulting in a dose of approximately 8.7 mg/kg/day (p.o). Animals were weaned with 3 days of 12.5 µg/mL, and then 3 days with 6.25 µg/mL, to allow for gradual recovery of endogenous corticosterone secretion.

2.8. Western Blot Analysis

At 35 days, each mouse was decapitated and its brain was quickly removed from the skull, briefly washed in ice-cold saline, and laid on a cooled (4°C) metal plate. The brain was rapidly dissected to separate the hippocampus and stored at -80°C until use. Brain samples were homogenized in 10 mL/g tissue ice-cold lysis buffer [50 mM Tris-HCl (pH 8.0) containing 159 mM NaCl, 50 mM EDTA, 1% Triton X-100, and protease/phosphatase inhibitor mixture] using a homogenizer (Physcotron; Microtec Co. Ltd., Chiba, Japan). Lysates were centrifuged at 12,000×g for 15 min at 4°C. Supernatants were collected and boiled for 5 min in SDS sample buffer (Wako). Equal amounts of protein were subjected to 10% SDS-PAGE gradient gel and then transferred to poly (vinylidene difluoride) membranes (Immobilon-P; Millipore, MA, USA). After blocking with Block Ace (Snow Brand Milk Products Co. Ltd., Tokyo, Japan) for 30 min, the membranes were incubated with primary antibody. The primary antibodies used were as follows: mouse anti-BiP antibody (BD Bioscience, CA, USA) for GRP78, mouse anti-KDEL antibody (Stressgen Bioreagents Limited Partnership, B.C., Canada) for GRP94, and mouse anti-actin antibody (Sigma-Aldrich, St. Louis, MO, USA). Subsequently, the membrane was incubated with the secondary antibody [goat anti-mouse (Pierce Biotechnology, IL, USA)]. The immunoreactive bands were visualized using Super Signal West Femto Maximum Sensitivity Substrate (Pierce Biotechnology) and then measured using LAS-4000 mini (Fujifilm, Tokyo, Japan).

2.9. Statistical Analysis

Statistical comparisons were made by Student's *t*-test using Statview version 5.0 (SAS Institute Inc., NC, USA), with $p < 0.05$ being considered statistically significant.

3. Results and Discussion

Real-time PCR was carried out to investigate whether the expression of ER stress response-related genes in the brain was changed by 6-hr restraint stress. In this study, we investigated the expression of GRP94, carleticulin, ER degradation-enhancing α-mannosidase-like protein (EDEM), protein kinase inhibitor of 58 kDa ($p58^{IPK}$), asparagines synthetase (ASNS), GRP78, ER-localized DnaJ 4 (ERdj4), and C/EBP homologous protein (CHOP). The expression of GRP78, GRP94, and calreticulin mRNA was significantly increased in the hippocampus, striatum, and cortex (**Figure 1**). In addition, there was significantly increased expression of $p58^{IPK}$ mRNA in the cortex, but not in the hippocampus or striatum.

We next investigated whether restraint stress affected the plasma concentrations of corticosterone, as previously reported. Immediately following the 6-hr restraint stress, significantly higher plasma corticosterone concentrations were found in stressed mice compared to unstressed mice. Seven days after the restraint stress, the plasma corticosterone recovered to the normal control level (**Figure 2**).

To clarify the mechanism of ER stress-related mRNA elevation, we artificially elevated the plasma concentrations of corticosterone in mice for 2 weeks and then measured the levels of ER stress-related proteins. In the corticosterone-treated animal model, the expression of GRP78 and GRP94 in the hippocampus was significantly increased compared to control levels (**Figure 3**).

Restraint stress is used widely to induce stress responses in animals, and it is known that a number of stresses, including restraint stress, can cause depression in animals. In the present study, we found that several ER stress-related genes were increased in the mouse hippocampus, striatum, and cortex after restraint stress.

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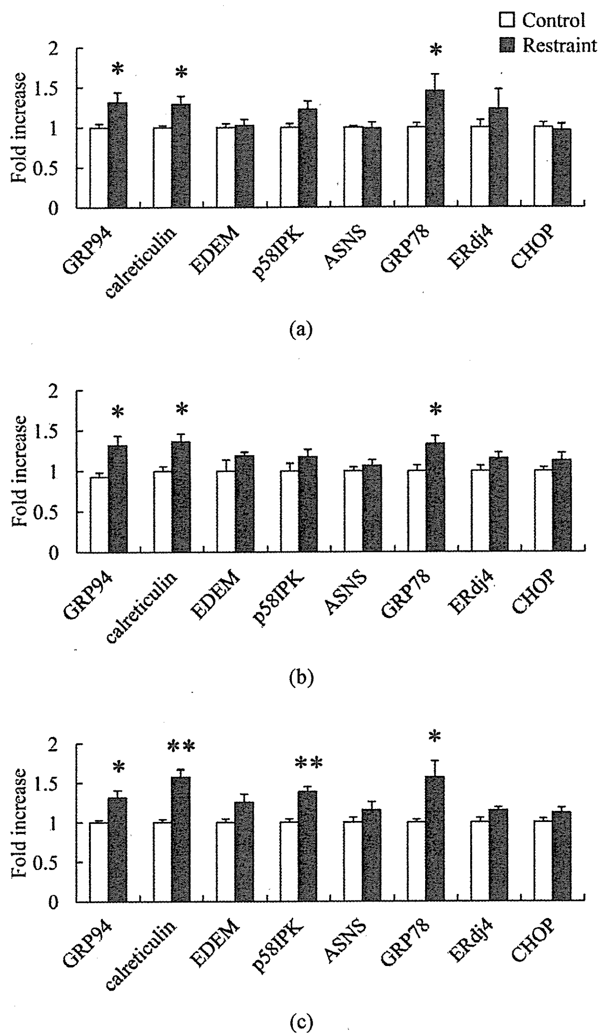

Figure 1. The expression mRNA of ER stress-related factors in the mouse brain after 6 hr restraint-stress. Mice were immobilized for 6 hr in a 50-mL perforated plastic tube. White and black bars represent the control group and the restraint group, respectively. Immediately after restraint, mice were killed and real-time PCR was performed on brain tissues from the (a) hippocampus, (b) striatum, and (c) cortex. Data represent means and S.E.M., n = 3 to 5. *p < 0.05, **p < 0.01 vs. control group. GRP94: the 94-kilodalton glucose regulated protein, EDEM: ER degradation-enhancing α-mannosidase-like protein, p58IPK: protein kinase inhibitor of 58 kilodalton, ASNS: asparagines synthetase, GRP78: the 78-kilodalton glucose regulated protein, ERdj4: ER-localized DnaJ 4, CHOP: C/EBP-homologous protein.

The significant increases in expression of GRP78, GRP94, and calreticulin agreed with the findings of a previous report of changes in the temporal cortex of subjects with major depression who died by suicide [11]. However, no study has yet specifically investigated expression changes of these genes in the hippocampus or the striatum in subjects with depression.

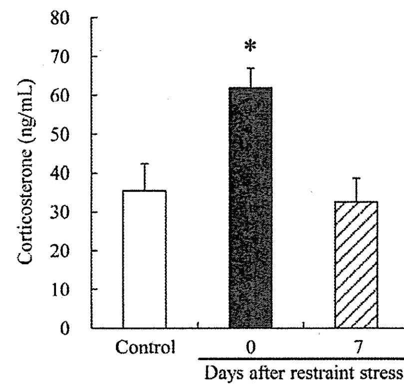

Figure 2. The effect of 6 hr restraint stress on the concentration of corticosterone in mouse plasma. Mice were immobilized for 6 hr. Immediately after restraint and 7 days later, blood samples were collected and concentration of plasma corticosterone was measured by ELISA. Restraint stress significantly increased the concentration of corticosterone in plasma. The corticosterone levels decreased to the normal control levels 7 days after restraint stress. Data represent means and S.E.M., n = 7. *p < 0.05 vs. control group.

GRP78, otherwise known as BiP, is one of the best-characterized ER chaperone proteins and is regarded as a classical marker of UPR activation. Overexpression of GRP78 has been reported to inhibit the upregulation of CHOP, which plays a key role in regulating cell growth and which has been implicated in apoptosis [19,20]. GRP94 and calreticulin are also ER chaperone proteins and show protective effects against ER stress [21]. The increase in these chaperones after restraint stress (**Figure 1**) may represent an attempt to oppose the toxic effect of prolonged stress and the high concentrations of glucocorticoid, such as corticosterone, on the brain. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, which controls glucocorticoid levels, has been reported in most depression patients and glucocorticoid level of depression patients was higher than those of normal ones [22-24]. In the mice in the present study, 6-hr restraint stress elevated the concentration of corticosterone in plasma, suggesting that restraint stress induced a response similar to depression.

Recently, corticosterone has been reported to exert immunostimulatory effects on macrophages *via* induction of ER stress [25]. Following corticosterone treatment, the glucocorticoid receptor (GR) binds onto B-cell lymphoma 2 (Bcl-2), a protein that affects cytochrome C and calcium release from mitochondria. Subsequently, this GR/Bcl-2 complex moves into mitochondria and regulates mitochondrial functions in an inverted "U"-shaped manner–i.e., a high dose treatment with corticosterone decreased levels of GRs and Bcl-2 in mitochondria and intracellular calcium was increased [26,27]. Substances

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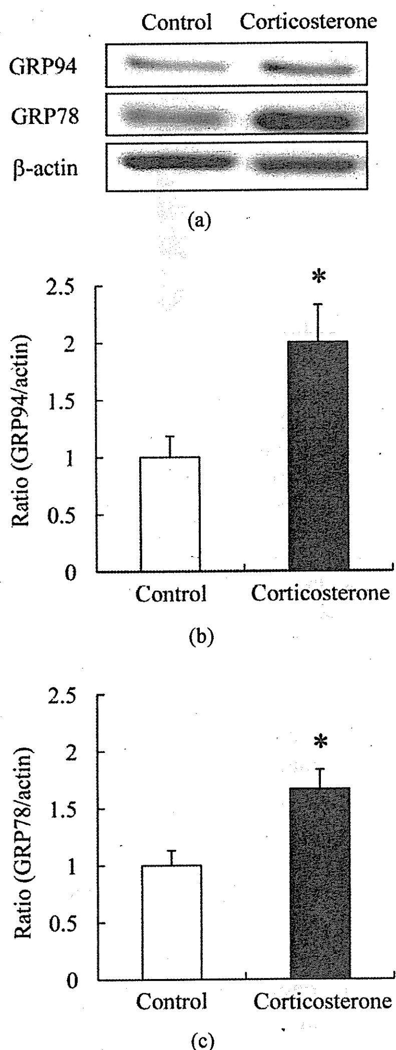

Figure 3. The expression of GRP78 and GRP94 in the hippocampus in a mouse model of chronic corticosterone induced depression. (a) Representative band images show immunoreactivities against GRP94, GRP78, and β-actin. (b) GRP78 expression was significantly increased by corticosterone exposure. (c) GRP94 expression was also increased by corticosterone exposure. Data represent means and S.E.M., n = 5 or 6. *p < 0.05 vs. control group.

that deplete the ER Ca^{2+} stores, such as thapsigargin, are widely used an ER stressors. Therefore, elevation of Ca^{2+} *via* GR may be sufficient for control of ER stress responses. In the present study, the restraint stress induced the expressions of only GRP78, GRP94, and calreticulin, but not other ER proteins. GRP78, GRP94, and calreticulin function as Ca^{2+} binding proteins [28]. Under the high concentration of corticosterone, the intracellular Ca^{2+} level might be higher, therefore, the expressions of GRP78, GRP94, and calreticulin might be increased.

Intracerebroventricular administration of thapsigargin has been reported to produce a depressant-like behavior [29]. A 14-days corticosterone treatment has also shown to induce depression symptoms in mice [18]. We used this animal model to investigate the effect of chronic elevation of corticosterone on ER stress responses in brain. As expected, significant increases in GRP78 and GRP94 proteins were observed in the hippocampus (**Figure 3**). The increase of GRP78 was consistent with the result of a previous report [30]. On the other hand, no change in these proteins was observed in the cortex (data not shown). Mineralocorticoid receptor (MR) and GR, which are the targets of corticosterone, are known to be well expressed in the hippocampus [31,32]. These reports, together with our findings, indicate that the hippocampus may be more sensitive to corticosterone exposure than are other brain regions. Many reports have referred to hippocampal atrophy in patients with depression [12,14]. In the cortex, it had been reported that chronic stress increased the caspase-3 positive neurons, in other words, exogenous stress was contributing to the cell apoptosis [15]. In our study, corticosterone exposure was performed for 2 weeks, but, in fact, long-term cortisol elevation has been observed in most depression patients. More extended corticosterone treatment may affect the expression of ER stress proteins in the cortex.

Recently, many experiments have focused on the relationship between depression and neurogenesis. Interestingly, ER stress also affects adult neurogenesis in the brain [33]. Brain-derived neurotrophic factor (BDNF), which promotes neurogenesis, is also known to inhibit neuronal cell death induced by ER stress [34]. These reports may also point to an involvement of ER stress in depression.

4. Conclusions

Restraint stress, which may contribute to depression in mice, may up-regulate the ER stress response *via* corticosterone elevation. This suggests the possibility of an ER stress involvement in the pathogenesis of stress-related depression disorders.

REFERENCES

[1] R. C. Kessler, P. Berglund, O. Demler, R. Jin, K. R. Me-rikangas and E. E. Walters, "Lifetime Prevalence and Age-of-onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication," *Archives of general psychiatry*, Vol. 62, No. 6, 2005, pp. 593-602. doi:10.1001/archpsyc.62.6.593

[2] D. Ron and P. Walter, "Signal Integration in the Endoplasmic Reticulum Unfolded Protein Response," *Nature reviews*, Vol. 8, No. 7, 2007, pp. 519-529.

[3] V. I. Rasheva and P. M. Domingos, "Cellular Responses to Endoplasmic Reticulum Stress and Apoptosis," *Apoptosis*, Vol. 14, No. 8, 2009, pp. 996-1007.

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