Ⅱ．研究成果の刊行に関する一覧表

1)書籍

著者名	書　名	出版者	発行年	総ページ数
長田賢一、御園生篤志、中野三穂、大友雅広、高橋清文、高橋美保、小川百合子、金井重人、田中大輔、貴家康男、朝倉幹雄.	精神科医からみた薬物療法	繊維筋痛症ハンドブック	2007	121-129

2)雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Kumagai Y, Kurokawa MS, Ueno H, Kayama M, Tsubota K, Nakatsuji N, Kondo Y, Ueno S, Suzuki N.	Induction of corneal epithelium-like cells from cynomolgus monkey embryonic stem cells and their experimental transplantation to damaged cornea.	Cornea			in press
Shimizu J, Yoshikawa H, Takada E, Hirosu C, Suzuki N.	Skewed helper T cell function in Behcet's disease.	Inflammation and Regeneration			in press
Kurokawa MS, Suzuki N.	Behcet's Disease.	Current Research in Immunology			in press
Hazama Y, Kurokawa MS, Chiba S, Tadokoro M, Imai T, Kondo Y, Nakatsuji N, Suzuki T, Hashimoto T, Suzuki N.	SDF1/CXCR4 Contributes to Neural Regeneration in Hemiplegic Mice with a Monkey ES-cell-derived Neural Graft.	Inflamation and Regeneration			in press
Kurokawa MS,Suzuki N.	Effect of nocotine on differentiation of vascular endothelial cells and mural cells derived from mouse embryonic stem cells.	St. Marianna Medical journal			in press
Osada K, Ogawa Y, Haga T, Nakano M, Tanaka D, Sasuga Y, Asakura M, Yamaguchi N.	Chronic trifluoperazine treatment increased P-glycoprotein in the rat brain.	St. Marianna Medical journal			in press
Nakano M, Osada K, Misonoo A, Fujiwara K, Takahashi M, Ogawa Y, Haga T, Kanai S, Tanaka D, Sasuga Y.	Fluvoxamine and sigma-1 receptor agonists dehydroepiandrosterone (DHEA)-sulfate induces the Ser473-phosphorylation of Akt-1 in PC12 cells.	Life Sciences			in press
Hatsugai M, Kurokawa MS, Kouro T, Nagai K, Arito M, Masuko K, Suematsu N, Okamoto K, Itoh F, Kato T.	Protein profiles of peripheral blood mononuclear cells are useful for differential diagnosis of ulcerative colitis and Crohn's disease.	J Gastroenterol			in press
Iizuka N, Okamoto K, Mastushita R, Kimura M, NagaiK, Arito M, Kurokawa MS, Masuko K, Suematsu N,HirohataS, Kato T.	Identification of autoantigens specific for systemic lupus erythematosus with central nerve system involvement.	Lupus			in press
Takahashi K, Miyoshi H, Otomo M, Osada K, Yamaguchi N, Nakashima H.	Suppression of dynamin GTPase activity by sertraline leads to inhibition of dynamin-dependent endocytosis.	Biochem Bio Res Comm	391	382-387	2010
T.Hiroi, S.Tatsunami, T.Yamamoto, R.Kuwabara, H.Kouyama, H.Matsui.	Preventative maintenanceof drainpipes in radio-isotope facility using flexible hose.	Radiation Saftey Management	8	14-18	2010
M.Kayama, MS Kurokawa, Y.Ueda, H. Ueno, Y. Kumagai , S.Chiba , E.Takada S.Ueno , M.Tadokoro, N. Suzuki.	Transfection with pax6 gene of mouse ES cells and subsequent cell cloning induced retinal neuron progenitors, including retinal ganglion cell-like cell, in vitro.	Ophthal Res	43(2)	79-91	2009
Fujii A, Chiba S, Takada E, Ueda Y, Shimizu J, Moroe Beppu, Suzuki N.	Generation of Spinal Motoneurons from Mouse Induced Pluripotent Stem Cells.	St. Marianna Medical journal	37(5)	327-336	2009
山口登.	老年期うつ病と認知症の共存と連続性	神経心理学	25(3)	177-181	2009
山口登.	高齢者のうつ病の症候学と診断学	Geriatic Medicine	47(11)	1427-1430	2009
山口登.	認知症の診断と治療	神経精神医学雑誌	111(1)	108-113	2009

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
長田賢一、宮本聖也、丸田智子、三宅誕実、中野三穂、山口登.	統合失調症に対するblonanserinの長期投与試験	臨床精神薬理	12(11)	2337-2351	2009
長田賢一.	Blonanserinの長期投与試験の臨床評価	臨床精神薬理	12(12)	2622-2629	2009
M. Katano, K. Okamoto, M. Arito, y. Kawakami, MS Kurokawa , N. Suematsu, S. Shimada,H. Nakamura , Y. Xiang , K. Masuko, K. Nihioka, K. Yudoh, T.Kato.	Implication of GM-CSF Induced neutrophil gelatinase-associated lipocalin in pathogenesis of rheumatoid arthritis revealed by proteome analysis.	Arthritis Res	11(1)	R3	2009
Nara K, Kurokawa MS, Chiba S, Yoshikawa H, Tsukikawa S, Matsuda T, Suzuki N.	Involvement of innate immunity in the pathogenesis of intestinal Behcet's disease.	Clin Exp Immunol	152	245-251	2008
T Hiroi, Y Okada, Y Masuda, R Kuwabara, H Kouyama, H Hasegawa, S Maruta, A Adachi, K Osada, M Asakura, N Yamaguchi, H Matsui.	Regulation of the tryptophan hydroxylase-2 gene promoter activity in immortalized serotonergic RN46A cells.	Fundam Clin Pharmacol,	22 (Suppl.2)	124-125	2008
三宅誕実, 宮本聖也, 竹内愛, 山田聡子, 田所正典, 大迫直子, 塚原さち子, 穴井己理子, 遠藤多香子, 諸川由実代, 山口登	統合失調症患者の認知機能障害に対するblonanserinの効果:risperidoneとの無作為化二重盲検比較	臨床精神薬理	11	315-326	2008
長谷川浩, 中村悦子, 朝倉幹雄, 山口登.	塩酸ドネペジル中断後に幻視体験が悪化したレビー小体型認知症の1例	精神医学	50(2)	197-199	2008
中野三穂, 宮本聖也, 関野敬子, 三宅誕実, 富永桂一郎, 新妻聖子, 竹内愛, 加藤文太, 山田浩史, 平山俊和, 山口登.	パーキンソニズムを呈する若年発症前頭側頭型認知症の1症例	精神科	13(3)	257-261	2008
渡部廣行, 山口登.	【高齢者のめまい診断におけるpitfall】高齢者の精神障害とめまい	ENTONI	87	130-135	2008
関野敬子, 山口登.	高齢者認知症の薬物治療の効果	日本医事新報	4380	94-95	2008
M. Otomo, K. Takahashi, H. Miyoshi, K. Osada, H. Nakashima, N. Yamaguchi.	Some selective serotonin reuptake inhibitors inhibit dynamin I GTPase.	Biol. Pharm Bull	88	1489-1495	2008
長田賢一、高橋清文、小川百合子、芳賀俊明、中野三穂、大友雅広,藤原圭亮、柳田拓洋、金井重人、田中大輔、貴家康男、長谷川浩、朝倉幹雄.	向精神薬の脳内濃度の調節するP糖蛋白質について	精神科	12(5)	434-439	2008
A. Misonoo, K. Osada, M. Nakano.	Chronic treatment with fluvoxaime stimulates phosophorylation of Ser473 anf Thr308 of AKT in the rat cerebral cortex	聖マリアンナ医科大学雑誌	36(3)	207-214	2008
長田賢一, 中野三穂, 大友雅広, 高橋清文.	P糖蛋白質-向精神薬の脳内濃度の調節について-	分子精神医学	8(4)	83-85	2008
S.Chiba , Y. M. Lee, W. Zhiu, C. R. Freed.	Noggin Enhances Dopamine Neuron Production from Humam Embryonic Stem Cells and Improves Behavioral Outcome After Transplantation into Parkinsonian Rats.	Stem Cells	26	2810-2820	2008

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Atoh K, Kurokawa MS, Yoshikawa H, Masuda C, Takada E, Kumagai N, Suzuki N.	Induction of melanocyte precursors from neural crest cells surrounding the neural tube like-structures developed in vitro using mouse ES cell culture.	Inflammation and Regeneration	27(1)	45-52	2007
Takenaga M, Ohta Y, Tokura Y, Hamaguchi A, Suzuki N, Nakamura M, Okano H, Igarashi R.	Plasma as a scaffold for regeneration of neural precursor cells after transplantation into rats with spinal cord injury.	Cell Transplantation	16(1)	57 -65	2007
Ueno H, Kurokawa MS, Kayama M, Homma R, Kumagai Y, Masuda C, Takada E, Tsubota K, Ueno S, Suzuki N.	Experimental transplantation of corneal epithelium-like cells induced by PAX6 gene transfection of mouse embryonic stem cells.	Cornea	26(10)	1220-1227	2007
黒川真奈絵、田子玲子、高田えりか、奈良和彦、鈴木登.	マウス胚性幹細胞由来血管内皮細胞および壁細胞の分化誘導	聖マリアンナ医科大学雑誌	35	143-149	2007
Kayama M, Kurokawa MS, Ueno H, Suzuki N.	Recent advances of corneal regeneration and possible application of embryonic stem (ES) cell derived corneal epithelial cells.	Clin Ophth	1(4)	373-382	2007
丸田智子、岡田容子、廣井朋子、増田陽子、桑原理恵、神山廣司、長谷川浩、足立淳、長田賢一、朝倉幹雄、山口登、松井宏晃.	ヒト脳型トリプトファン水酸化酵素遺伝子発現調節機構:転写抑制配列の同定と機能解析	日本神経精神薬理学会雑誌	27	322	2007
杉山恒之、中村悦子、山口登.	期待される新規作用機序の抗認知症薬	臨床精神薬理	10(11)	2019ー2026	2007
中村悦子、柳田浩、山口登.	アルツハイマー病の重症度と海馬体積および海馬左右比との関係	老年精神医学雑誌	18	1217ー1223	2007
荻野あずみ、杉山恒之、山口登.	アルツハイマー型認知症の記憶・認知機能障害に対するdonepezilの効果内容と効果出現の関連因子について	臨床精神薬理	10(1)	93-101	2007
山口登.	塩酸ドネペジルの使い方-家族、介護者への説明の重要性の観点から	CLINICIAN	558(54)	430-449	2007
長谷川浩、朝倉幹雄、中野三穂、長田賢一、山口登.	前頭側頭型認知症にパーキンソン症候群を合併した一例	精神医学	49	1129ー1132	2007
Hasegawa H, Osada K, Yamaguti N.	Schizophrenia.	Super Review	25	1-10	2007
長田賢一.	うつ病患者におけるSSRI, SNRI惹起性性機能障害への対策	精神科治療学	22(11)	1265-1270	2007
長田賢一、富永桂一郎、岡 寛、西岡久寿樹、磯村達也、中村郁朗、高橋忍、小島綾子.	日本語版Fibromyalgia Impact Quesionnarire (JFIQ) の開発: 言語的妥当性を担保した翻訳版の作成	臨床リウマチ	20	19-28	2007
金井重人、朝倉幹雄、中野三穂、田中大輔、菱沼拓児、御園生篤志、長田 賢一.	慢性ストレスによる聴性驚愕反応のストレス感受性亢進の持続	日本神経精神薬理学雑誌	27	13-18	2007

3)学会発表

発表者氏名	論文タイトル名	発表学会名	発表年度
千葉俊明、谷口泰造、鈴木登.	認知症モデルマウスにおける移植治療への検討	第9回日本再生医療学会	2010
Shimizu J, Suzuki N, Kaneko F, Kaneko S.	Accelerated expression of interleukin-23 and SMAD2 mRNA in Behcet`sdisease.	The 9th Worla Congress on Inflammation	2009
Shimizu J, Yoshikawa H, Suzuki N, Kaneko F, Kaneko S.	Accelerated expression of TGF-beta receptor and Smad2 mRNA in Behçet's disease.	第39回日本免疫学会総会・学術集会	2009
Taniguchi T, Kadoyama K., Takenokuchi M, Chiba S, Suzuki N, Matsumoto A, Matsuyama S.	PARKINSONISM INDUCED BY TAUOPATHY: ANALYSIS OF SJLB MICE.	The 22nd Biennial Meeting of the ISN/APSN Joint Meeting	2009
Taniguchi T, Chiba S, Kadoyama K, Nagata D, Tanaka S, KawaKami T, Suzuki N, Sagara EJ, Matsuyama S.	Parkinsonism in Tauopathy: analysis of SJLB mice.	日本動物学会	2009
嘉山真紀、千葉俊明、上野宏樹、熊谷悠太、高田えりか、加藤由理子、田所衛、上野聡樹、鈴木登.	pax6遺伝子導入によるマウス胚性幹細胞の網膜神経前駆細胞への分化誘導	第8回日本再生医療学会総会	2009
高橋清文、三好洋、大友雅広、長田賢一、山口登、中島秀喜.	Sertaraline inhibit dynamin-dependent endocytosis by suppressing dynamin GTPase activities.	, 第32回日本分子生物学会	2009
Y. Ogawa, H. Matsui, K. Osada, T. Haga, M. Nakano, K. Takahashi, K. Fujiwara, T.Yanagida, S. Kanai, D. Tanaka, Y. Sasuga, M. Asakura.	Milnacipram of antidepressant bound with fluorescence-NBD-F passed and accumulated into HT22 cell.	40th Annual Meeting of Neuroscience	2009
K. Osada, Y. Ogawa, H. Matsui, T. Haga, M. Nakano, K. Takahashi, K. Fujiwara, T.Yanagida, S. Kanai, D. Tanaka, Y. Sasuga, M. Asakura.	Chronic trifluoperazine treatment increased P-glycoprotein in the rat brain.	40th Annual Meeting of Neuroscience	2009
T. Haga, Y. Ogawa, H. Matsui, K. Takahashi, D. Tanaka, S. Kanani, Y. Sasuga, M. Nakano, T. Yanagida, K. Fujiwara, M. Asakura, K. Osada.	Validation of a fluorescence-NBD-F high-throughput method for the cellular noradrenaline uptake activity.	40th Annual Meeting of Neuroscience	2009
岡本一起、永井宏平、有戸光美、黒川真奈絵、増子佳世、末松直也、遊道和雄、礒橋文秀、加藤智啓.	ビタミンB6酵素の転写を促進する核内受容体コアクティベーター(MTI-II)のホモログタンパク質(prothymsin)の活性	日本ビタミン学会第61回大会	2009
有戸光美、黒川真奈絵、増子佳世、岡本一起、永井宏平、末松直也、加藤智啓.	「アセチル化」プロテオミクスによる関節リウマチ(RA)関連分子の探索	日本プロテオーム機構第7回大会	2009
初谷守朗、黒川真奈絵、紅露剛史、永井宏平、有戸光美、増子佳世、末松直也、岡本一起、伊東文生、加藤智啓.	末梢血単核球のプロテオーム解析による潰瘍性大腸炎クローン病の鑑別診断	日本プロテオーム機構第7回大会	2009
飯塚進子、岡本一起、有戸光美、永井宏平、黒川真奈絵、増子佳世、末松直也、廣畑俊成、加藤智啓.	中枢神経ループスにおける抗神経細胞抗体の対応抗原の検出	日本プロテオーム機構第7回大会	2009
高桑由希子、黒川真奈絵、大岡正道、永井宏平、有戸光美、増子佳世、末松直也、岡本一起、尾崎承一、加藤智啓.	顕微鏡的多発血管炎患者血清ペプチドの網羅的探索	日本プロテオーム機構第7回大会	2009
深澤雅彦、岡本一起、中村学、永井宏平、有戸光美、黒川真奈絵、増子佳世、末松直也、肥塚泉、加藤智啓.	めまいモデルラット前庭代償期における小脳片葉プロテオーム解析	日本プロテオーム機構第7回大会	2009
金城永幸、小板橋賢一郎、Yang Xiang、永井宏平、黒川真奈絵、岡本一起、有戸光美、増子佳世、遊道和雄、安田隆、末松直也、木村健二郎、加藤智啓.	血清ペプチドミクス解析によるIgA腎症の診断マーカーの探索	日本プロテオーム機構第7回大会	2009
安藤敬、永井宏平、近田正英、岡本一起、黒川真奈絵、増子佳世、有戸光美、末松直也、小林俊也、加藤智啓、幕内晴朗.	プロテオミクスによる腹部大動脈瘤形成機序の解明	第50回日本脈管学会総会	2009

発表者氏名	論文タイトル名	発表学会名	発表年度
Arito M, Kurokawa M, Masuko K, Okamoto K, Nagai K, Suematsu N, Kato T.	Acetyl-Proteomics for the Investigation of Pathological Molecules in Rheumatoid Arthritis.	HUPO 8th Annual World Congress	2009
Nagai K, Kaneshiro N, Xiang Y, Kurokawa M, Okamoto K, Arito M, Masuko K, Yudoh K, Yasuda T, Suematsu N, Kimura K, Kato T.	Comprehensive analysis of short peptides in sera from patients with IgA nephropathy.	HUPO 8th Annual World Congress	2009
Fukasawa M, Okamoto K, Nakamura M, Nagai K, Arito M, Kurokawa M, Masuko K, Suematsu N, Koizuka I, Kato T.	Proteomics of Cerebellar Floccules dunng Vestibular Compensation of A Rat Vertigo Model.	HUPO 8th Annual World Congress	2009
Hatsugai M, Kurokawa M, Kouro T, Nagai K, Arito M, Masuko K, Suematsu N, Okamoto K, Ito F, Kato T.	Protein Profiles of Peripheral Blood Mononucleocytes are Useful for Discrimination of Ulcerative Colitis from Crohn's Disease.	HUPO 8th Annual World Congress	2009
有戸光美, 松尾光祐、黒川真奈絵、永井宏平、岡本一起、増子佳世、末松直也、加藤智啓.	関節リウマチ関連分子アネキシンA7の機能解析	第82回日本生化学会大会	2009
初谷守朗、黒川真奈絵、紅露剛史、永井宏平、有戸光美、増子佳世、末松直也、岡本一起、伊東文生、加藤智啓.	末梢血単核球のプロテオーム解析による潰瘍性大腸炎とクローン病の鑑別診断	第82回日本生化学会大会	2009
高桑由希子、黒川真奈絵、大岡正道、永井宏平、有戸光美、増子佳世、末松直也、岡本一起、尾崎承一、加藤智啓.	顕微鏡的多発血管炎患者血清ペプチドの網羅的探索	第82回日本生化学会大会	2009
岡本一起、末松直也、増子佳世、黒川真奈絵、有戸光美、永井宏平、遊道和雄、加藤智啓.	新規の核内レセプター・コアクティベーター(MTI-II)の立体構造と転写促進活性	第82回日本生化学会大会	2009
深澤雅彦、岡本一起、中村学、永井宏平、有戸光美、黒川真奈絵、増子佳世、末松直也、肥塚泉、加藤智啓.	片側内耳破壊後の前庭代償におけるラット小脳片葉タンパク質のプロテオーム解析	第82回日本生化学会大会	2009
永井宏平、金城永幸、Yang Xiang、黒川真奈絵、岡本一起、有戸光美、増子佳世、遊道和雄、安田隆、末松直也、木村健二郎、加藤智啓.	多変量解析OPLS-DAを用いたIgA腎症の血清ペプチドマーカーの検索	第82回日本生化学会大会	2009
Takakuwa Y, Kurokawa MS, Ooka S, Nagai K, Arito M, Masuko K, Suematsu N, Okamoto K, Ozaki S, Kato T.	Comprehensive Analyses of Serum Peptides in Microscopic Polyangiitis.	American College of Rheumatology 71th scientific meeting	2009
Iizuka N, Okamoto K, Mastushita R, Kimura M, Nagai K, Arito M, Kurokawa MS, Masuko K, Suematsu N, HirohataS, Kato T.	Identification of autoantigens specific for systemic lupus erythematosus with central nerve system involvement.	American College of Rheumatology 71th scientific meeting	2009
嘉山真紀、黒川真奈絵、上野宏樹、熊谷悠太、千葉俊明、上野聰樹、鈴木登.	マウス胚性肝細胞由来神経細胞を用いた視神経損傷モデルマウスに対する移植	第7回日本再生医療学会総会	2008
上野宏樹、黒川真奈絵、嘉山真紀、熊谷悠太、本間龍介、坪田一男、上野聰樹、鈴木登.	マウス胚性肝細胞由来角膜上皮様細胞による角膜損傷モデルの移植と治療	第7回日本再生医療学会総会	2008
熊谷悠太、黒川真奈絵、上野宏樹、嘉山真紀、坪田一男、中辻憲夫, 仁藤新治、上野聰樹、鈴木登.	カニクイザル胚性幹細胞を用いた角膜上皮様細胞への分化誘導と角膜移植への対応	第7回日本再生医療学会総会	2008
藤井厚司、千葉俊明、黒川真奈絵、清水弘之、別府諸兄、鈴木登.	マウスを用いた下肢切断・再接着モデルの検討	第7回日本再生医療学会総会	2008
K. Nara, MS. Kurokawa, T. Matsuda, N.Suzuki.	microphage may be responsible for the development of neuro-Bechet's disease.	第52回日本リウマチ学会総会・第17回国際リウマチシンポジウム	2008
H. Ueno, MS. Kurokawa, Y. Kumagai, M. Kayama, R. Homma, C. Masuda E. Takada, K. Tubota, S. Ueno, N. Suzuki.	Development of Corneal Epithelial Cell Sheets Derived from Mouse Embryonic Stem (ES) Cells Transfected withPAX6 Gene.	The Association for Research in vision and Ophthalmology 2008	2008

発表者氏名	論文タイトル名	発表学会名	発表年度
M. Kayama, M. S. Kurokawa, Y. Ueda, H. Ueno, Y. Kumagai, C. Masuda, E. Takada, R. Tago, S. Ueno, N. Suzuki.	Subretinal Transplantation of Embryonic Stem Cell Derived Neuroretinal Cells to Mice With Optic Nerve Injury.	The Association for Research in vision and Ophthalmology 2008	2008
Y. Kumagai, MS. Kurokawa, H. Ueno, M. Kayama, K. Tsubota, N. Nakatsuji, Y. Kondo, S. Ueno, N. Suzuki.	Induction of Corneal Epithelium-Like Cells From Non Human Primate Embryonic Stem Cells and Their Experimental Transplantation to Damaged Cornea.	The Association for Research in vision and Ophthalmology 2008	2008
奈良和彦、黒川真奈絵、松田隆秀、鈴木登.	神経ベーチェット病における病変部TNFα産生細胞の検討	第29回日本炎症・再生医学会	2008
千葉俊明、加藤由理子、谷口泰造、鈴木登.	N279Kタウ遺伝子を発見する認知症デルマウスSJLBの組織学的検討	第29回日本炎症・再生医学会	2008
上野宏樹、黒川真奈絵、嘉山真紀、熊谷悠太、本間龍介、坪田一男、上野聰樹、鈴木登	マウス胚性幹細胞由来角膜上皮様細胞による角膜損傷モデルの移植治療	第29回日本炎症・再生医学会	2008
嘉山真紀、黒川真奈絵、上野宏樹、熊谷悠太、千葉俊明、上野聰樹、鈴木登	マウス胚性幹細胞由来網膜前駆細胞を用いた視神経損傷モデルマウスに対する移植の検討	第29回日本炎症・再生医学会	2008
熊谷悠太、黒川真奈絵、上野宏樹、嘉山真紀、坪田一男、中辻憲夫、仁藤新治、上野聰樹、鈴木登.	カニクイザルES細胞を用いた角膜上皮様細胞の分化誘導及び角膜移植への応用	第29回日本炎症・再生医学会	2008
N. Suzuki, K Nara, M.S. Kurokawa, S. Chiba , T Matsuda, H. Yoshikawa, J . Shimizu.	Innate immunity and Th1 cell responses in the pathogenesis of intestinal Behcet's disease.	MEDICAL -EXPO 2008 in APLAR's World	2008
M. Okazaki, I. Utagawa, C. Hshimoto, A. Tanaka, E . Nakamura, K. Sekino, K.Tominaga, M. Tadokoro, S. Tsukahara, N. Yamaguchi.	Hypercholesterolemia in alzh-eimer' s disease reduces the effects of donepezil hydrochloride.	Collegium international neuropsychopharmacologi-cum, Munichi Germany	2008
田中絢子、岡崎味音、中村悦子、富永桂一朗、関野敬子、荻野あずみ、穴井理巳子、、塚原さち子、田所典、山口登.	認知機能と海馬体積及び大脳皮質下病変との関係	第23回老年精神医学会	2008
長田賢一.	抗うつ薬を含めた精神科薬物療法	第2回繊維筋痛症研究会	2008
K. Osada, M. Nakano, A. Misono, S. Kanai, D. Tanaka, M. Ootomo, Y. Sasuga, K. Takahashi, Y. Ogawa, T.Haga, M. Asakura.	Antidepressants induces phosphorylation of Akt in PC12.	Neuroscience 2008 meeting	2008
Y.Ogawa, H.Matsui, K.Osada, T.Haga, M.Nakano, A.Misonoo, M.Ootomo, K.Takahashi, K.Fujiwara, T.Yanagida, S.Kanai, D.Tanaka, Y.Sasuga, M.Asakura.	Fluorescence-based high-throughput method for milnacipran in vivo cells.	Neuroscience 2008 meeting	2008
T.Haga, K.Osada, Y.Ogawa, M.Nakano, A.Misonoo, M.Ootomo, K.Takahashi, K.Fujiwara, T.Yanagida, S.Kanai, D.Tanaka, Y.Sasuga, M.Asakura.	Validation of a fluorescence-based high-throughput method for the cellular serotonin uptake activity.	Neuroscience 2008 meeting	2008
飯塚進子、広畑俊成、岡本一起、増子佳世、末松直也、黒川真奈絵、松下礼子、加藤智啓.	プロテオミクスを用いた、ループス精神病における抗神経細胞抗体の認識エピトープの検出	日本プロテオーム機構第6回大会	2008
金城永幸、岡本一起、有戸光美、黒川真奈絵、増子佳世、末松直也、木村健二郎、加藤智啓.	IgA腎症の扁桃を用いた病因抗原のプロテオーム探索	日本プロテオーム機構第6回大会	2008
片野雅淑、松尾光祐、黒川真奈絵、有戸光美、増子佳世、末松直也、岡本一起、加藤智啓.	関節リウマチ滑膜細胞のリン酸化プロテオーム解析	日本プロテオーム機構第6回大会	2008
飯塚進子、廣畑俊成、岡本一起、黒川真奈絵、増子佳世、末松直也、松下礼子、加藤智啓.	ループス精神病における抗神経細胞抗体の対応抗原のプロテオーム解析	第6回北里疾患プロテオーム研究会	2008
金城永幸、岡本一起、黒川真奈絵、有戸光美、増子佳世、末松直也、木村健二郎、加藤智啓.	IgA腎症の血清と特異的に反応する扁桃タンパク質の網羅的解析 —病因抗原のプロテオーム探索—	第6回北里疾患プロテオーム研究会	2008

発表者氏名	論文タイトル名	発表学会名	発表年度
Kurokawa MS, Matsuo K, Nakamura H, Masuko K, Okamoto K, Kato T.	Arthritis- Inducible Protein, Annexin VII: High Phosphorylation in Rheumatoid Arthritis.	HUPO 2008 7th World Congress	2008
Kurokawa MS, Arito M, Masuko K, Suematsu N, Okamoto K, Suzuki N, Kato T.	Comprehensive analysis of protein expression in peripheral blood mononucleocytes from patients with Behcet's disease.	MEDICAL-EXPO 2008 in APLAR's World	2008
岡本一起、末松直也、増子佳世、黒川真奈絵、有戸光美、遊道和雄、加藤智啓.	細胞内導入配列を付加した核内受容体コアクティベーター(MTI-II)の細胞内への取り込みと転写促進活性	第31回日本分子生物学会第81回日本生化学会大会合同大会	2008
有戸光美、松尾光祐、末松直也、増子佳世、黒川真奈絵、岡本一起、加藤智啓.	関節リウマチ関連分子アネキシンVIIの機能解析	第31回日本分子生物学会・第81回日本生化学会大会合同大会	2008
深澤雅彦、岡本一起、中村学、有戸光美、黒川真奈絵、増子佳世、末松直也、肥塚泉、加藤智啓.	片側内耳破壊後の前庭代償におけるラット小脳片葉タンパク質のプロテオーム解析	第31回日本分子生物学会・第81回日本生化学会大会合同大会	2008
Kurokawa MS, Suzuki N, Kato T.	ベーチェット病抹消血単核球における発現蛋白の網羅的検討	第37日本免疫学会総会・学術集会	2007
奈良和彦、黒川真奈絵、松田隆秀、鈴木登.	神経ベーチェット病における炎症性サイトカイン産生とその責任細胞の検討	第37日本免疫学会総会・学術集会	2007
嘉山真紀、黒川真奈絵、上野宏樹、熊谷悠太、千葉俊明、田所衛、上野聰樹、鈴木登.	pax6遺伝子導入によるマウス胚性幹細胞の網膜神経節細胞への分化誘導	第10回日本組織工学会	2007
上野宏樹、黒川真奈絵、嘉山真紀、熊谷悠太、本間龍介、坪田一男、上野聰樹、鈴木登.	マウス胚性幹細胞より分化誘導した角膜上皮様細胞の特徴と角膜損傷モデルへの移植	第10回日本組織工学会	2007
熊谷悠太、黒川真奈絵、上野宏樹、嘉山真紀、坪田一男、中辻憲夫、仁藤新治、上野聰樹、鈴木登.	霊長類胚性幹細胞を用いた角膜上皮細胞の分化誘導と移植治療への応用	第10回日本組織工学会	2007
間淑郎、黒川真奈絵、奈良和彦、千葉俊明、池田律子、仁藤新治、中辻憲夫、橋本卓雄、鈴木登.	片麻痺モデルマウスにおける霊長類胚性幹(ES)細胞由来神経細胞移植の有用性	第66回日本脳神経外科学会総会	2007
嘉山真紀、黒川真奈絵、上野宏樹、熊谷悠太、千葉俊明、田所衛、上野聰樹、鈴木登.	Pax6遺伝子導入によるマウス胚性幹細胞の選択的網膜神経節前駆細胞への分化誘導	第28回日本炎症・再生医学会	2007
熊谷悠太、黒川真奈絵、上野宏樹、嘉山真紀、坪田一男、中辻憲夫、仁藤新治、上野聰樹、鈴木登.	カニクイザル胚性幹細胞の角膜上皮細胞への分化誘導及び移植治療への応用実験	第28回日本炎症・再生医学会	2007
上野宏樹、黒川真奈絵、嘉山真紀、熊谷悠太、本間龍介、坪田一男、上野聰樹、鈴木登.	マウス胚性幹細胞より分化誘導した角膜上皮様細胞の特性と角膜損傷モデルへの移植治療	第28回日本炎症・再生医学会	2007
間淑郎、黒川真奈絵、池田律子、仁藤新治、中辻憲夫、近藤靖、長田乾、橋本卓雄、鈴木登.	カニクイザルES細胞からの運動神経分化誘導と脳損傷マウスへの移植応用	第28回日本炎症・再生医学会	2007
黒川真奈絵、鈴木登.	マウス胚性幹細胞からの血管内皮細胞の誘導	第28回日本炎症・再生医学会	2007
Suzuki N.	Immune and inflammatory responses in Behcet's Disease.	Japan Korea Joint Meeting on Behcet's	2007
黒川真奈絵、鈴木登.	胚性幹細胞由来血管内皮細胞および壁細胞の分化・増殖・維持に与える禁煙の影響	第22回 平成18年度喫煙財団助成研究発表会	2007
Kayama M, Kurokawa MS, Ueda Y, Ueno H, Kumagai Y, Masuda C, Takada E, Ueno S, Tadokoro M, Suzuki N.	Induction of Differentiation Into Retinal Ganglion Cells of Mouse Es Cells by Pax6 Gene Transfection.	The Association for Research in vision and Ophthalmology 2007	2007
Kumagai Y, Kurokawa MS, Ueno H, Kayama M, Tsubota K, Nakatsuji N, Nito S, Ueno S, Suzuki N.	Induction of Corneal Epithelium-Like Cells From Cynomorgus Monkey Embrionic Stem Cells and Their Experimental Transplantation to Damaged Cornea.	The Association for Research in vision and Ophthalmology 2007	2007
Ueno H, Kurokawa MS, Kumagai Y, Kayama M, Homma R, Masuda C, Takada E, Tsubota K, Ueno S, Suzuki N.	Characterization of Corneal Epithelium Like Cells Induced by Pax6 Gene Transfection of Mouse Embryonic Stem Cells.	The Association for Research in vision and Ophthalmology 2007	2007

発表者氏名	論文タイトル名	発表学会名	発表年度
黒川真奈絵、松田隆秀、鈴木登.	神経ベーチェット病の病態形成における炎症性サイトカインの役割	第51回日本リウマチ学会総会・学術集会	2007
奈良和彦、黒川真奈絵、金子栄、吉川英志、松田隆秀、鈴木登.	ベーチェット病患者の病変局所におけるTLRを介したTh1優位の免疫応答	第51回日本リウマチ学会総会・学術集会	2007
野中信宏、黒川真奈絵、池島秀明、松田隆秀、鈴木登.	神経ベーチェット病の病態形成における炎症性サイトカインの役割	第104回日本内科学会総会・講演会	2007
上野宏樹、黒川真奈絵、嘉山真紀、熊谷悠太、本間龍介、坪田一男、上野聰樹、鈴木登.	マウス胚性幹細胞より分化誘導した角膜上皮様細胞の角膜損傷モデルへの移植	第6回日本再生医療学会総会	2007
嘉山真紀、黒川真奈絵、上野宏樹、熊谷悠太、田所衛、上野聰樹、鈴木登.	Pax6遺伝子導入によるマウス胚性幹細胞の網膜神経節前駆細胞への分化誘導	第6回日本再生医療学会総会	2007
熊谷悠太、黒川真奈絵、上野宏樹、嘉山真紀、本間龍介、坪田一男、上野聰樹、鈴木登.	カニクイザル胚性幹細胞からの角膜上皮細胞の分化誘導と移植治療への応用	第6回日本再生医療学会総会	2007
間淑郎、黒川真奈絵、池田律子、仁藤新治、中辻憲夫、橋本卓雄、鈴木登.	カニクイザルES細胞からの運動神経分化誘導と脳損傷マウスへの移植応用	第6回日本再生医療学会総会	2007
Hiroi T, Okada T, Masuda T, Kuwabara R, Kouyama H, Hasegawa H, Maruta S, Adachi A, Asakura M, ,Yamaguchi N, Matsui H.	Regula- tion of the tryptophan hydroxylase-2 gene promoter activity in immortalized seroto- nergic RN46A cells.	37th Annual Meeting for Neuroscience Society	2007
Osada K, Misonoo A, Nakano M, Ootomo M, Ogawa Y, Takahashi K, Takahashi M, Kanai S, Tanaka D, Asakura M, Inoue Y, Yamaguchi N.	Chronic trifluoperazine treatment increased P-glycoprotein in the rat brain.	37rd Annual Meeting of Neuroscience	2007
Nakano M, Misonoo A, Osada K, Kanai S, Tanaka D, Ootomo M, Sasuga M, Takahashi K, Takahashi M, Ogawa Y, Asakura M.	Fluvoxamine induces phosphorylation of Akt in PC12.	37rd Annual Meeting of Neuroscience	2007
長田賢一、御園生篤志、中野三穂、高橋清文、小川百合子、高橋美保、金井重人、田中大輔、貴家康男、宮本聖也、朝倉幹雄、山口登.	Trifluoperazineのラット慢性投与後の脳内P糖蛋白質への影響	第26回躁うつ病の薬理・生化学的研究懇話会	2007

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Fluvoxamine and sigma-1 receptor agonists dehydroepiandrosterone (DHEA)-sulfate induces the Ser^{473}-phosphorylation of Akt-1 in PC12 cells

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ABSTRACT

Aims: The expression of brain-derived neurotrophic factor (BDNF) may be a downstream target of a variety of antidepressant treatments, and selective serotonin reuptake inhibitors (SSRIs) are used clinically for the treatment of depression. BDNF binds to and activates tyrosine kinases receptor (TrkB) to exert its effects. TrkB, after activation by ligands, stimulates phosphoinositide 3-kinase (PI3K). The downstream target of PI3K is Akt-1, a serine-threonine kinase. BDNF has signaling through the PLC-IP_3/Ca^{2+} pathway. Furthermore, the PLC-$\gamma/IP_3/Ca^{2+}$ pathway is regulated by the sigma-1 receptors. Here, we examined whether fluvoxamine (FLV) activated Akt-1 and increased phosphorylation of Akt-1 via sigma-1 receptor in PC12 cells.

Main methods: We examined the effect of the SSRI, FLV and BDNF on the phosphorylation levels of serine-threonine kinase Akt-1 in PC12 cells using immunoblotting techniques.

Key findings: Treatment with 10 μM and 100 μM FLV of PC12 cells stimulated a 2.4- and 3.8-fold maximal increase in Ser^{473}-phosphorylated Akt-1 levels at 40 min, respectively. Treatment with 50 ng/ml BDNF also stimulated Ser^{473} -phosphorylated Akt-1 by 2.6-fold with a maximal increase at 5 min. In addition, the phosphorylation induced by FLV and BDNF was blocked by LY294002, a selective inhibitor of PI3K. The sigma-1 receptor agonists dehydroepiandrosterone (DHEA)-sulfate also stimulated a 2.1-fold increase in the level of Ser473-phosphorylated Akt-1.

Significance: This study demonstrates that fluvoxamine treatment rapidly increased phosphorylation of Akt-1. And BDNF activated Akt-1 phosphorylation by the TrkB/PI3K/Akt-1 pathway. We conclude that the phosphorylation of Akt-1, downstream of PI3K, was the key to their antidepressant effects.

Introduction

Mood disorders are among the most prevalent, recurrent and disabling of all mental illnesses (American Psychiatric Association 1994) Selective serotonin reuptake inhibitors (SSRIs) are representative antidepressant agents. These drugs increase the levels of serotonin, norepinephrine, glutamate, and brain-derived neurotrophic factor (BDNF) by blocking the reuptake of these neurotransmitters at presynaptic terminals (Duman et al. 1997; Skolnick 1999). Although an increase in the monoamine levels occurs soon after drug administration, the efficacy of antidepressants only emerges gradually after several weeks of continuous administration (Nestler et al. 2002a). Therefore, in recent years, research directed at sites beyond the level of the monoamines and receptors has examined the potential post-receptor mechanisms involved in the actions of antidepressant treatments.

Several lines of evidence suggest that the expression of BDNF may be a downstream target of a variety of antidepressant treatments (Altar 1999; Coyle and Duman 2003; Duman et al. 2000; Nestler et al. 2002b). BDNF is a 27-kDa polypeptide that binds to and activates tyrosine kinase receptor (TrkB) to exert their effects (Schlessinger and Ullrich 1992; Barbacid 1995; Weiss et al. 1998). TrkB, once activated by ligands, stimulates the expression of PI3K (Araki et al. 2000). The downstream target of phosphoinositide 3-kinase (PI3K) is Akt-1, a serine-threonine kinase, also referred to as protein kinase B (PKB). Akt-1 has a pleckstrin homology domain in its N-terminus. Activation of Akt-1 by PI3K is induced when the homology domain binds to phosphatidylinositol-3,4,5-trisphosphate and phosphatidylinositol-3,4-bisphosphate, which leads to the translocation of Akt-1 to the

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plasma membrane (Franke et al. 1995,1997a,b). The phosphorylation of Akt-1 on Thr^{308} and Ser^{473} by the PI-dependent kinases PDK1 and PDK2 is also important for the activation of Akt-1 (Marte and Downward 1997). The expression of BDNF is decreased by exposure to stress (Smith et al. 1995a,b; Nibuya et al. 1999), suggesting that BDNF might be involved in the pathophysiology of stress-related mood disorders.

Several animal and human studies suggest that BDNF may be involved in the actions of antidepressants. For example, infusions of BDNF directly into the rat midbrain area or hippocampus mimics the effects of antidepressants in behavioral models of depression, such as learned helplessness and the forced swim test (Siuciak et al. 1997; Shirayama et al. 2002; Hashimoto et al. 2004). These results suggest that antidepressant drugs might partially exert their effects through an up-regulation of BDNF, and that BDNF is a key molecule involved both in the pathophysiology of depressive disorders and perhaps in the pharmacological action of antidepressants as well (Shirayama et al. 2002).

BDNF has signaling through the PLC-$\gamma/IP_3/Ca^{2+}$ pathway. Furthermore, the PLC-$\gamma/IP_3/Ca^{2+}$ pathway is regulated by sigma-1 receptors (Hayashi et al. 2001). Yagasaki et al. reported that BDNF-stimulated PLC-γ activation and the intracellular $Ca2^{2+}$ and BDNF signaling is modulated by sigma-1 receptors (Yagasaki et al. 2006). Nishimura T. et al. reported that the FLV, with sigma-1 receptor agonist, and sigma-1 receptor agonists DHEA-sulfate were shown to potentiated nerve-growth factor (NGF)-induced neurite outgrowth in PC 12 cells (Nishimura et al. 2008).

Here, we examined the activation of thePI3K/Akt-1 signaling pathway with FLV, BDNF and DHEA-sulfate through the protein and phosphorylation levels of Akt-1 in PC12 cells using immunoblotting techniques.

Materials and methods

Drugs

Recombinant human BDNF and dehydroepiandrosterone (DHEA)-sulfate were obtained from Sigma-Aldrich (St. Louis, MO, USA), and LY294002 was obtained from Cell Signaling Technology (Beverly, MA, USA).

Cell culture

PC12 cells were maintained in 75-cm^2 flasks in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 μg of streptomycin/ml, 100 U of penicillin/ml and 250 μg/ml of amphotericin B, and incubated at 37 °C with a 5% CO_2 humidified atmosphere. The culture medium was replaced twice a week with fresh medium. The stock culture was routinely sub-cultured at a ratio of 1:5 at weekly intervals.

Treatments

Before each experiment, the cells were detached using 5 mM EDTA in Hank's balanced buffer (HBS) and seeded in 12- or 6-well plates (coated with poly-D-Lysine, 10 μg/ml) at a density of $3–6 \times 10^5$ cells/well in 2% serum medium for 24 h. Thereafter, to ensure a low degree of phosphorylation of Akt-1 in the controls all experiments were serum starved for 2 h before the desired reagents were added. The cells were treated to study the effects of FLV and BDNF on the Ser^{473}-phosphorylation of Akt-1. Alternatively, the cells were pretreated with LY294002 (50 μM, 30 min) followed by stimulation with FLV (100 μM, 10–80 min) and BDNF (50 ng/ml, 5 min). PC 12 cells were treated with sigma-1 receptor agonists DHEA-sulfate (10 μM, 24 h) in free-serum medium. LY294002 and DHEA-sulfate were dissolved in 0.2% dimethyl sulfoxide.

Western blotting

PC12 cells were washed with ice-cold PBS three times and then the protein was extracted with RIPA buffer (25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 0.1 μg/ml DTT, 0.2 nM okadaic acid (OA), and 10 μg/ml each of PMSF, calyculin A, and $NaVO_3$), and placed on ice for 30 min. Thereafter, the extracts were centrifuged at 15,000 rpm at 4 °C for 15 min. Aliquots containing 20 μg of protein from each sample, as determined extemporarily following instructions for the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Inc, CA, USA), were mixed with Laemmli sample buffer (2% SDS), placed in a boiling water bath for 10 min, and processed for SDS-PAGE. Proteins were separated in 10% SDS polyacrylamide gels (Perfect NT Gel; DRC, JAPAN) and the separated proteins were transferred to nitrocellulose membranes. The membranes were blocked with 50 mM Tris-HCL buffer (pH 7.5) containing 10% skim milk for 1 h. Immunoblots were probed with antibodies to anti-phospho-Akt-1 (Ser^{473}) at a dilution of 1:500 (Cell Signaling Technology, Beverly, MA, USA), anti-Akt 1 at a dilution of 1:1000 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or anti-β-Actin at a dilution of 1:1000 (Cell Signaling Technology, Beverly, MA, USA) overnight at 4 °C in the same buffer; and then incubated with horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) at a dilution of 1:1000 for 1 h. Proteins were detected using the Enhanced Luminol Chemiluminescence system (GE Healthcare, Bio-Science, NJ, USA) and direct exposure to the LAS-3000 imaging system with a full-frame-capture CCD camera (FUJIFILM Corporation, Tokyo, Japan). The immunoblots were quantified using a densitometric analyzer (ATTO CS Analyzer 2.0, Tokyo, Japan).

MTT assay

After incubation of the PC12 cells with the experimental reagents for 24 h, cell viability was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay in which the yellow tetrazolium base compound is reduced by viable cells to a blue formazan product. The original medium was removed from the 96-well plates, and the plates were incubated at 37 °C in the presence of RPMI 1640 medium supplemented with 1% FBS containing 0.5 mg/ml MTT. A 100-μl aliquot of acid-isopropanol (0.04 M hydrochloric acid) was then added to each well, and the plates were incubated at 37 °C overnight to dissolve the formazan that had formed in the wells. Reduced MTT was measured using a spectrophotometer (Wallac ARVO HTS 1420 Multilabel Counter, Perkin Elmer Life Sciences, Turku, Finland) at a wavelength of 570 nm. The value for each treatment group was converted to a percentage of the control.

Statistical analysis

The results are expressed as mean ± S.E.M. of three or more experiments performed in duplicate. One-way ANOVA with Newman–Keuls test was used to establish statistical significance, set at $p<0.05$.

Results

Effects of FLV on MTT reduction of PC12 cells

Because the cytotoxicity of FLV in PC12 cells had not yet been established, we examined the concentration-dependent effect of FLV on the viability of cells by quantifying the reduced product of MMT in PC12 cells. One-way ANOVA revealed that the viability of the PC12 cells was reduced significantly after treatment for 24 h with 400 μM FLV, but was not affected by lower concentrations of this drug ($p<0.001$; Fig. 1). That is, FLV is not cytotoxic at concentrations equal to or less than 200 μM.

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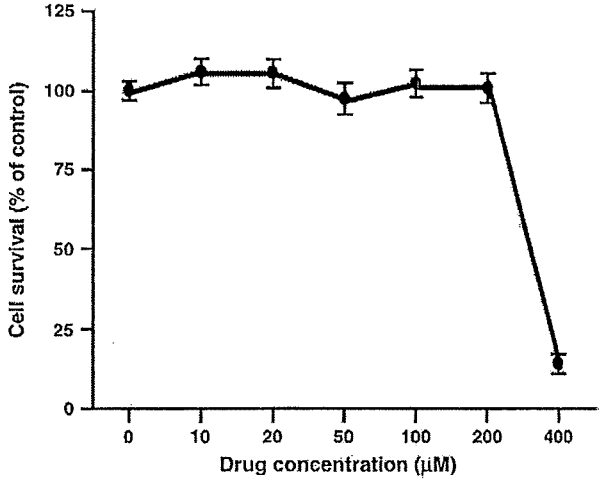

Fig. 1. Effect of FLV on MMT reduction. PC12 cells were treated with FLV for 24 h. The MTT reduction assay was carried out as described in the Methods section. Data are expressed as a percentage of the corresponding control value, which was set at 100%. The results were compared by ANOVA. ($n=12$) (***$p<0.001$).

We therefore decided to observe the phosphorylation of Akt-1 induced by 100 μM FLV.

FLV- and BDNF-stimulated activation of Akt-1

To examine the FLV- and BDNF-stimulated activation of Akt-1 in PC12 cells, we performed Western blotting using anti-phospho (Ser^{473}) Akt-1 antibody. Treatment of PC12 cells with 10 μM FLV stimulated a 2.4-fold increase in the level of Ser^{473}-phosphorylated Akt-1 at 40 min ($p<0.05$) (Fig. 2). The phosphorylation of Akt-1 increased a maximum of 3.8 fold with 100 μM FLV. The phosphorylation of this kinase was observed within 20 min of treatment and reached a maximal level at 40 min ($p<0.001$). The level of Ser^{473}-phosphyrylated Akt-1 remained elevated even at 60 min. The levels of total Akt-1 protein and β-Actin protein were unaffected (Fig. 3).

Treatment with 50 ng/ml BDNF was found to produce similar results to those produced by FLV with regard to the phosphorylation of Akt-1. This phosphorylation was observed within 2.5 min of treatment and was maximally increased at 5 min by 2.6-fold ($p<0.01$). The level of phosphorylated Akt-1 remained elevated even at 40 min. The levels of total Akt-1 and β-Actin protein were unaffected (Fig. 4).

FLV and BDNF-induced phosphorylation of Akt-1 is mediated by the PI3K pathway

PC12 cells were then pretreated with the PI3K inhibitor, LY294002 (50 μM, 30 min), and stimulated with FLV (100 μM, 40 min) and BDNF (50 ng/ml, 5 min). The FLV-treated cells without LY294002 induced an increase in the levels of Ser^{473}-phosphorylation of Akt-1 ($p<0.001$). On the other hand, pretreatment with LY294002 blocked the FLV-induced Ser^{473}-phosphorylation of Akt-1 ($p<0.001$). Similarly, the BDNF-treated cells without LY294002 showed an increase in the Ser^{473} phosphorylation of Akt-1 ($p<0.001$), and LY294002 blocked the BDNF-induced Ser^{473}-phosphorylation of Akt-1 ($p<0.001$). No significant difference was observed between the effects of FLV and BDNF on the Ser^{473}-phophorylation of Akt-1 ($p>0.05$). The protein level of Akt-1 and β-Actin was unchanged from the control level after treatment with FLV and BDNF (Fig. 5).

Sigma-1 receptor agonists DHEA-sulfate stimulated activation of Akt-1

PC12 cells were then pretreated with the sigma-1 receptor agonists DHEA-sulfate (10 μM, 24 hr). DHEA-sulfate stimulated a 2.1-fold increase in the level of Ser^{473}-phosphorylated Akt-1

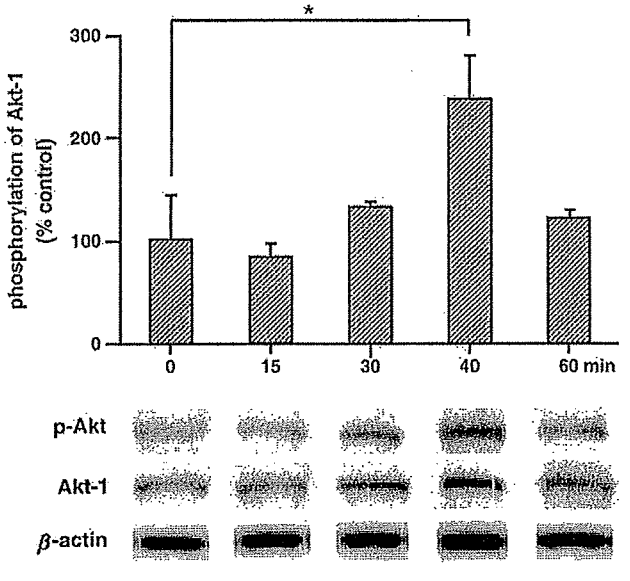

Fig. 2. Effect of 10 μM FLV on Akt-1 (Ser^{473}) phosphorylation and total Akt-1 protein levels. 10 μM FLV stimulates the phosphorylation of Akt-1 in PC12 cells. PC12 cells were incubated with 10 μM FLV for the indicated times and lysed. The supernatant was analyzed by immunoblotting with an antibody against phospho-specific (Ser^{473}) Akt-1, total Akt-1 and β-Actin. The densitometric data were expressed as a percentage of the corresponding control value, which was set at 100%. The results are expressed as the mean ± S.E.M. Representative immunoblots from experiments repeated three times are shown ($n=4$) (*$p<0.05$).

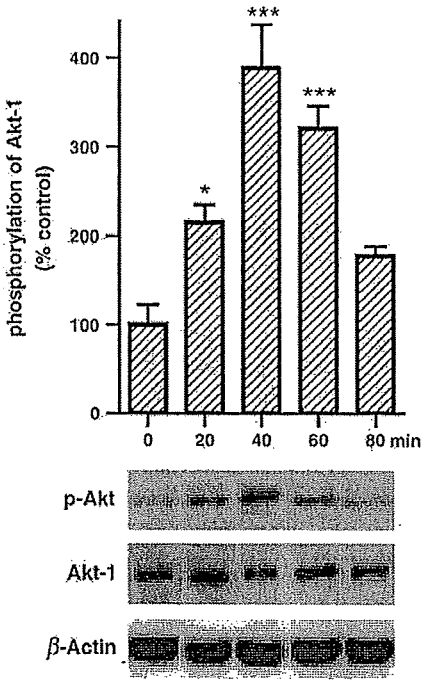

Fig. 3. Effect of 100 μM FLV on Akt-1 (Ser^{473}) phosphorylation and the total Akt-1 protein levels. PC12 cells were incubated with 100 μM FLV for the indicated times and lysed. The supernatant was analyzed by immunoblotting with an antibody against phospho-specific (Ser^{473}) Akt-1, total Akt-1 and β-Actin. The densitometric data were expressed as a percentage of the corresponding control value, which was set at 100%. The results are expressed as the means ± S.E.M. Representative immunoblots from experiments repeated three times are shown ($n=7$) (*$p<0.05$; ***$p<0.001$).

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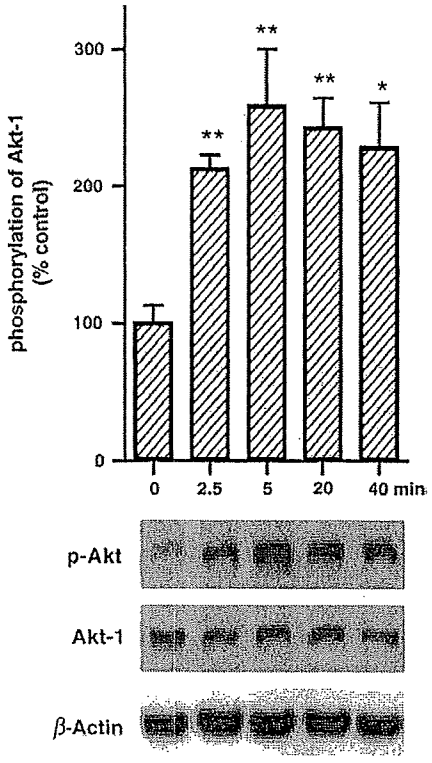

Fig. 4. Effect of BDNF on Akt-1 (Ser^{473}) phosphorylation and the total Akt-1 protein levels. BDNF stimulated the phosphorylation of Akt-1 in PC12 cells. PC12 cells were incubated with 50 ng/ml BDNF for the indicated times and lysed. The supernatant was analyzed by immunoblotting with antibodies against phospho-specific (Ser^{473}) Akt-1, total Akt-1 and β-Actin. The densitometric data were expressed as a percentage of the corresponding control value, which was set at 100%. The results are expressed as the mean ± S.E.M. Representative immunoblots from experiments repeated three times are shown ($n=6$) (*$p<0.05$; **$p<0.01$).

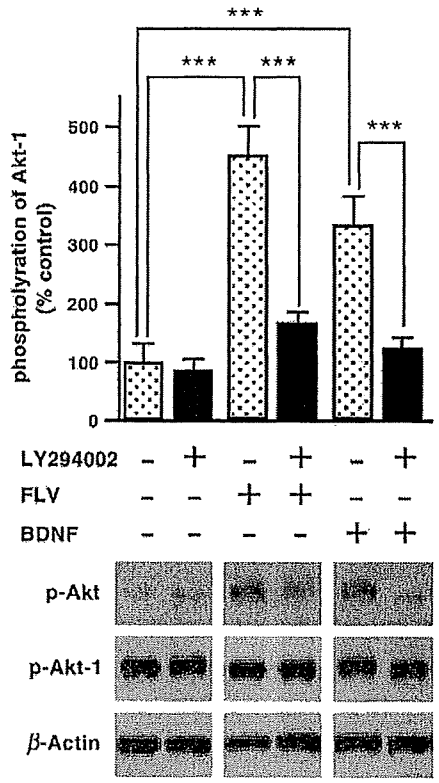

Fig. 5. Effect of FLV and BDNF on the inhibition of Akt-1. Effects of FLV and BDNF on Akt-1 (Ser^{473}) phosphorylation and the total Akt-1 protein levels treated with or without the PI3K inhibitor, LY294002. The cultures were pretreated for 30 min, where indicated with 50 μM LY294002, and then 100 μM FLV was administered for 40 min and 50 ng/ml BDNF was administered for 5 min. The cultures not treated with LY294002 were treated with vehicle (0.2% dimethyl sulfoxide). The supernatant was analyzed by immunoblotting with antibodies against phospho-specific (Ser^{473}) Akt-1, total Akt-1 and β-Actin. The densitometric data were expressed as a percentage of the corresponding control value, which was set at 100%. The results are expressed as the mean ± S.E.M. The representative immunoblots from experiments repeated three times are shown ($n=6$) (***$p<0.001$).

($p<0.001$); however, the levels of total Akt-1 and β-Actin protein were unaffected (Fig. 6).

Discussion

Our results demonstrate that the administration of both the antidepressant FLV and BDNF in PC12 cells rapidly increased Akt-1 phosphorylation in a time-dependent manner, as shown by the immunoblots.

The phosphorylation of Akt-1 has been reported to be critically dependent on PI3K activity. High levels of basal Akt-1 activity are most likely caused by the presence of growth factors in the culture medium, and the removal of serum induced a rapid depletion of Akt-1 activity (Elzbieta and De-Maw 1999). We measured the phosphorylation of Akt-1 in PC12 cells incubated with 2% serum medium for 24 h, after which the serum was removed for 2 h, 4 h, 12 h, and 24 h. The degree of phosphorylation of Akt-1 was no changed from 2 h to 24 h (data not shown) and we concluded that the phosphorylation of Akt-1 induced by serum was not affected by removal of the serum for 2 h. PC12 cells were under the same physiologic conditions as those observed at 2 h. Therefore, all of our experiments were performed after 2 h of serum starvation. Because phosphorylation of Akt-1 is crucial to the activity of this kinase, our results demonstrate that FLV and BDNF affected the phosphorylation state of Akt-1 in PC12 cells incubated with serum-free medium.

The activity of BDNF is also directly influenced by antidepressant treatment. For example, chronic, but not acute, antidepressant treatment up-regulates BDNF mRNA in the rat brain (Nibuya et al. 1995; Russo-Neustadt et al. 2000). Chen et al. reported that the levels of BDNF were higher in post-mortem hippocampi obtained from antidepressant-treated subjects than in those from untreated subjects (Chen et al. 2001). Saarelainen et al. showed that antidepressants (imipramine and fluoxetine) acutely increase TrkB signaling in the cerebral cortex and that this signaling is required to determine the behavioral effects caused by antidepressants. They suggested that antidepressants exert their effects, at least in part, through the actions of endogenous BDNF (Saarelainen et al. 2003).

SSRIs appear to affect the activity of serotonin transporters (SERTs), but this has not yet been definitively demonstrated. Recently, the effects of citalopram, one of the SSRIs, have been assessed in a forced swimming test using mice in which SERTs were knocked down via siRNA. In that report, the effects of the SSRI were not changed after SERT knockdown (Thakker et al. 2005). Therefore, SSRIs may not act via SERTs. Furthermore, Nishimura et al. showed that, in an experiment with PC12 cells, the activity of neurite outgrowth due to SSRIs (FLV, sertraline and paroxetine) occurs because SSRIs activate IP3 receptors via sigma-1 receptors (Nishimura et al. 2008). Moreover, Zhou et al. reported that SSRIs such as FLV exhibit a suppressive activity on plasma membrane monoamine transporters (PMATs), which are not SERTs, but are also important sites of action for SSRIs (Zhou et al. 2006). Therefore, it is currently difficult to conclude that SSRIs act only on SERT. Some studies have reported that antidepressants have a similar effect to BDNF, and both antidepressants and BDNF produced antidepressant effects. Nibuya et al. reported that when K252a, an inhibitor of TrkB receptors, is

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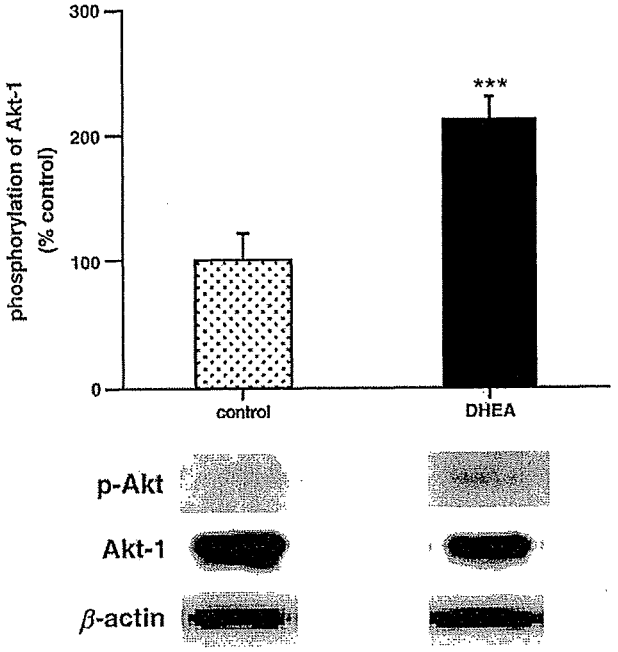

Fig. 6. Effect of the increase in the Ser^{473}- phosphorylation of Akt-1 by sigma-1 receptor agonists DHEA-sulfate. The cultures were treated for 24 hr with 10 μM DHEA-sulfate or vehicle (0.2% dimethyl sulfoxide) in serum-free medium. The supernatant was analyzed by immunoblotting with antibodies against phospho-specific (Ser^{473}) Akt-1, total Akt-1 and β-Actin. The densitometric data were expressed as a percentage of the corresponding control value, which was set at 100%. The results are expressed as the mean ± S.E.M. The representative immunoblots from experiments repeated three times are shown ($n=6$) (***$p<0.001$).

administered with BDNF concurrently, the effects of BDNF are abolished, suggesting that the antidepressant activity of BDNF exerts its effects through TrkB receptors (Nibuya et al. 1999). However, no reports have yet described antidepressants involved after the activation of TrkB.

BDNF activated Akt-1 phosphorylation by the TrkB/ PI3K/Akt-1 pathway. We confirmed the present results with BDNF-induced activation of Ser^{473}-phosphorylated Akt-1 by PI3K. Furthermore, BDNF signals through the PLC-γ/IP_3/Ca^{2+} pathway regulated by sigma-1 receptors (Yagasaki et al. 2006). Y. Yagasaki et al. reported that BDNF-stimulated PLC-γ activation and the intracellular $Ca2^{2+}$ and BDNF signaling are modulated by sigma-1 receptors; FLV, with sigma-1 receptor agonism, enhanced BDNF-induced glutamate release and increased intracellular Ca^{2+} in cultured cortical neurons (Yagasaki et al. 2006). Nishimura T. et al. reported that the FLV and sigma-1 receptor agonists DHEA-sulfate were shown to potentiated NGF-induced neurite outgrowth by the PLC-γ/IP_3/Ca^{2+} pathway in PC 12 cells (Nishimura, T. PLoS ONE 3(7), 1–9, 2008). In the present study, FLV and DHEA-sulfate increased in the level of Ser^{473}-phosphorylated Akt-1. Therefore, we considered that FLV activated Akt-1 and increased phosphorylation of Akt-1 via the sigma1 receptor in a manner similar to that shown by DHEA-sulfate in PC12 cells.

Up-regulation of BDNF has been suggested to contribute to the action of antidepressants. BDNF activated Akt-1 by the TrkB/PI3K/Akt-1 pathway, and FLV activated Akt-1 by the PLC-γ/IP_3/Ca^{2+}/Akt-1 pathway via the sigma-1 receptor. In the present study, Akt-1 phosphorylation induced by FLV was blocked by pretreatment with LY294002, a highly selective inhibitor of PI3K as shown in the Fig. 5. Therefore, we have shown firstly presented that FLV activated Ser^{473}-phosphorylated Akt-1 by PI3K in PC12 cells. Because BDNF activated Akt-1 phosphorylation by the TrkB/PI3K/Akt-1 pathway and FLV activated Akt-1 phosphorylation by the PLC-γ/IP_3/Ca^{2+}/Akt-1 pathway via the sigma-1 receptor, we conclude that the phosphorylation of Akt-1, the downstream of PI3K, was the key enzyme to their antidepressants effects.

Conclusions

Treatment with fluvoxamine increased in Ser^{473}-phosphorylated Akt-1 levels on PC12 cells. Treatment with BDNF also stimulated Ser^{473} -phosphorylated Akt-1. In addition, the phosphorylation induced by fluvoxamine and BDNF was blocked by LY294002, a selective inhibitor of PI3K. Sigma-1 receptor agonists DHEA-sulfate also increased Ser^{473} -phosphorylated Akt-1. This study is the first to demonstrate that fluvoxamine treatment rapidly increases the phosphorylation of Akt-1.

References

Altar CA. Neurotrophins and depression. Trends in Pharmacological Sciences 20 (2), 59–61, 1999.
American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington, DC, 1994.
Araki T, Yamada M, Ohnishi H, Sano S, Uetsuki T, Hatanaka H. Shp-2 specifically regulates several tyrosine-phosphorylated proteins in brain-derived neurotrophic factor signaling in cultured cerebral cortical neurons. Journal of Neurochemistry 74 (2), 659–668, 2000.
Barbacid M. Structural and functional properties of the TRK family of neurotrophin receptors. Annals of the New York Academy of Sciences 7, 442–458, 1995.
Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biological Psychiatry 50 (4), 260–265, 2001.
Coyle JT, Duman RS. Finding the intracellular signaling pathways affected by mood disorder treatments. Neuron 38 (2), 157–160, 2003.
Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. Archives of General Psychiatry 54 (7), 597–606, 1997.
Duman RS, Malberg J, Nakagawa S, D'Sa C. Neuronal plasticity and survival in mood disorders. Biological Psychiatry 48 (8), 732–739, 2000.
Elzbiet C, De-Maw C. Lithium activates the serine/ threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. Proceedings of the National Academy of Science 96 (6), 8745–8750, 1999.
Franke TF, Yang SI, Chan TO, Datta K, Kazlauskas A, Morrison DK, Kaplan DR, Tsichlis PN. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. Cell 81 (5), 727–736, 1995.
Franke TF, Kaplan DR, Cantley LC. PI3K: downstream action blocks apoptosis. Cell 88 (4), 435–437, 1997a.
Franke TF, Kaplan DR, Cantley LC, Toker A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3, 4-bisphosphate. Science 275 (5300), 665–668, 1997b.
Hashimoto K, Shimizu E, Iyo M. Critical role of brain-derived neurotrophic factor in mood disorders. Brain Research Reviews 45 (2), 104–114, 2004.
Hayashi T, Hayashi T, Su TP. Regulating ankyrin dynamics: roles of sigma-1 receptors. Proceedings of the National Academy of Sciences of the United States of America 98, 491–496, 2001.
Marte BM, Downward J. PKB/Akt: connecting phosphoinositide 3-kinase to cell survival and beyond. Trends in Biochemical Sciences 22 (9), 355–358, 1997.
Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 34 (1), 13–25, 2002a.
Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L, Zalcman S. Preclinical models: status of basic research in depression. Biological Psychiatry 52 (6), 503–528, 2002b.
Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. The Journal of Neuroscience 15 (11), 7539–7547, 1995.
Nibuya M, Takahashi M, Russell DS, Duman RS. Repeated stress increases catalytic TrkB mRNA in rat hippocampus. Neuroscience Letters 267 (2), 81–84, 1999.
Nishimura T, Ishima T, Iyo M, Hashimoto K. Potentiation of nerve growth factor-induced neurite outgrowth by fluvoxamine: role of sigma-1 receptors, IP3 receptors and cellular signaling pathways. PLoS ONE 3 (7), 1–9, 2008.
Russo-Neustadt AA, Beard RC, Huang YM, Cotman CW. Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience 101 (2), 305–312, 2000.
Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P, Castrén E. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. The Journal of Neuroscience 23 (1), 349–357, 2003.
Schlessinger J, Ullrich A. Growth factor signaling by receptor tyrosine kinases. Neuron 9 (3), 383–391, 1992.
Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. The Journal of Neuroscience 22 (8), 3251–3261, 2002.

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Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacology, Biochemistry, and Behavior 56 (1), 131–137, 1997.

Skolnick P. Antidepressants for the new millennium. European Journal of Pharmacology 375 (1–3), 31–40, 1999.

Smith MA, Makino S, Kim SY, Kvetnansky R. Stress increases brain-derived neurotrophic factor messenger ribonucleic acid in the hypothalamus and pituitary. Endocrinology 136 (9), 3743–3750, 1995a.

Smith MA, Makino S, Kvetnansky R. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. The Journal of Neuroscience 15 (3 Pt 1), 1768–1777, 1995b.

Thakker DR, Natt F, Huske D, van der Putten H, Maier R, Hoyer D, Cryan JF. siRNA-mediated knockdown of the serotonin transporter in the adult mouse brain. Molecular Psychiatry 10(8), 782-789, 714, 2005.

Weiss ER, Maness P, Lauder JM. Why do neurotransmitters act like growth factors? Perspectives on Developmental Neurobiology 5 (4), 323–335, 1998.

Yagasaki Y, Numakawa T, Kumamaru E, Hayashi T, Su TP, Kunugi H. Chronic antidepressants potentiate via sigma-1 receptors the brain-derived neurotrophic factor-induced signaling for glutamate release. The Journal of Biological Chemistry 281 (18), 12941–12949, 2006.

Zhou M, Enge K, Wang J. Evidence for significant contribution of a newly identified monoamine transporter (PMAT) to serotonin uptake in the human brain. Biochemical Pharmacology 73 (1), 147–154, 2006.

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Suppression of dynamin GTPase activity by sertraline leads to inhibition of dynamin-dependent endocytosis

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ABSTRACT

Dynamin (Dyn) 1 plays a role in recycling of synaptic vesicles, and thus in nervous system function. We previously showed that sertraline, a selective serotonin reuptake inhibitor (SSRI), is a mixed-type inhibitor of Dyn 1 with respect to both GTP and L-α-phosphatidyl-L-serine (PS) *in vitro*, and we suggested that it may regulate the neurotransmitter transport by modulating synaptic vesicle endocytosis *via* inhibition of Dyn 1 GTPase. Here, we investigated the effect of sertraline on endocytosis of marker proteins in human neuroblastoma SH-Sy5Y cells and HeLa cells. Sertraline inhibited endocytosis in both cell lines. Western blotting showed that SH-Sy5Y expresses Dyn 1 and Dyn 2, while HeLa expresses only Dyn 2. GTPase assay showed that sertraline inhibited Dyn 2 as well as Dyn 1. Therefore, the effect of sertraline on endocytosis was mediated by Dyn 2, at least in HeLa cells, as well as by Dyn 1 in cell lines that express it. Moreover, the inhibition mechanism of transferrin (Tf) uptake by sertraline differed from that in cells expressing Dyn 1 K44A, a GTP binding-defective variant, and sertraline did not interfere with the interaction between Dyn 1 and PS-liposomes.

Introduction

Dynamin (Dyn) has GTPase activity and plays a critical role in clathrin- and caveolae-dependent endocytosis [1,2]. A major role of Dyn GTPase activity in endocytosis is to produce a mechanical force for membrane fission during clathrin-coated vesicle budding, either by constriction or expansion of the collar surrounding the neck of the invaginated vesicle [3].

Mammalians have three Dyn isoforms with different tissue distributions [4,5]. Dyn 1 is only expressed in neurons and has been implicated in presynaptic vesicle recycling [4]. It is thought to play a role in the clathrin-dependent endocytotic pathway at neuronal synapses [6,7]. Dyn 1 has four functional domains: an N-terminal GTPase domain, a pleckstrin homology domain (PHD), a proline/arginine-rich domain (PRD), and a GTPase effector domain [3,8–11]. L-α-Phosphatidyl-L-serine (PS) or phosphatidylinositol-4,5-bisphosphate binds the PHD of Dyn [8,9], stimulates the GTPase activity, and induces cooperative helix assembly [10,12]. Moreover, after deletion of the PHD, Dyn shows elevated GTPase activity independently of those lipids [8,9]. PRD of Dyn binds to Src homology 3 (SH3) domain-containing proteins, such as amphiphysin [13], and the complex is necessary for adaptation of clathrin [14]. PRD peptide disrupts the interaction between Dyn and amphiphysin and inhibits endocytosis [15].

The K44A variant of Dyn lacks GTPase activity owing to defects in both GTP binding and hydrolytic activity [2,16]. As a result, overexpression of the K44A variant inhibits clathrin-dependent endocytosis in neuronal cells [2,16,17].

The GTPase activities of Dyn 1 and 2 are inhibited by cationic surfactants such as myristyl trimethyl ammonium bromide [18], and by 3-(2-chloro-10*H*-phenothiazin-10-yl)-*N*,*N*-dimethylpropan-1-amine (chlorpromazine), which is an antipsychotic [19]. We previously reported that (1*S*)-*cis*-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalenamine (sertraline) inhibits the GTPase activity of Dyn 1 [20]. Our previous results indicated that the inhibition of Dyn 1 GTPase by sertraline may regulate the endocytic pathway at neuronal synapses.

Sertraline is a selective serotonin reuptake inhibitor (SSRI) [21]. The serotonin transporter is proposed to modulate a variety of brain functions, including mood, anxiety and sleep, by the

Abbreviations: SSRI, selective serotonin reuptake inhibitor; His_6, polyhistidine segment 6 residues in length; Dyn, dynamin; Dyn-His_6, dynamin 1 with a His_6 tag fused to the C-terminus; PHD, pleckstrin homology domain; PRD, proline/arginine-rich domain; SH3, Src homology 3; PS, L-α-phosphatidyl-L-serine; Pi, orthophosphate; SPR, surface plasmon resonance; CTB, cholera toxin subunit B; Tf, transferrin; CPZ, chlorpromazine; DAPI, 4′,6-diamidino-2-phenylindole; CHC, clathrin heavy chain

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elimination of the neurotransmitter serotonin from the synaptic cleft [22]. Depressive disorder has been postulated to be associated with continuously low levels of serotonin [23]. However, our finding that some SSRIs inhibit Dyn 1 GTPase [20] was unexpected, and furthermore, the relationship between inhibition of Dyn 1 GTPase and regulation of endocytosis by sertraline is still unknown. In this study, we further examined the mechanism of sertraline's inhibitory action on endocytosis.

Materials and methods

Materials. Sources of materials were: restriction enzymes (Takara Bio); pEGFP-C1 (Clontech); pET 21a (Merck); Alexa Fluor®555 conjugate-CTB, Alexa Fluor®633 conjugate-Tf, Prolong Gold and Lipofectamin™2000 (Invitrogen); L-α-phosphatidyl-L-serine (Sigma Aldrich); Dyn 1 antibody (Epitomics); Dyn 2 (C-18) antibody, clathrin HC (C-20) antibody, rabbit anti-goat antibody and goat anti-mouse antibody (Santa Cruz Biotechnology); β-actin rabbit antibody (Cell Signaling); sertraline, citalopram and chlorpromazine (Sigma); ECL Western blotting detection reagents (GE Healthcare).

Cloning and construction of expression plasmids. Dyn 2 gene (gi:87299636) was amplified by PCR using appropriate primers. The amplified gene was ligated into the NdeI and SalI sites in pET 21a to create an expression vector for Dyn 2 *wt* bearing a polyhistidine segment 6 residues in length (His_6 tag) fused to the C-terminus (Dyn2-His_6). Cloning and construction of pETDyn1, an expression vector for Dyn 1 *wt* bearing a His_6 tag fused to the C-terminus (Dyn-His_6), were reported previously [20]. Dyn 1 *wt* gene was subcloned into pBluescript2SK(+) from pETDyn1 to reconstruct expression plasmids for mammalian cells. The subcloned vector was named pBlueDyn1*wt*. Site-directed mutagenesis was performed by PCR to create an expression vector for Dyn K44A bearing a His_6 tag fused to the C-terminus (Dyn K44A-His_6). This was digested with NdeI and XhoI, and ligated into the same sites in both pET 21a and pBlueDyn1*wt*, affording pETDyn1K44A and pBlueDyn1K44A, respectively. A pETDyn1 plasmid was digested with BstBI and BspEI, blunt-ended and ligated to delete 530–550 amino acid residues (Dyn ΔPHD-His_6), affording pETDyn1ΔPHD. pBlueDyn1 plasmid was digested with BglII and EcoRI, and ligated into the same sites in pEGFP-C1 to create an expression vector for Dyn 1 linked at the N-terminus to GFP, designated pEGFP-Dyn1. pEGFP-Dyn1K44A plasmid was constructed similarly to pEGFP-Dyn1.

Expression and purification of Dyn. Expression and purification of Dyn-His_6 were reported previously [20]. DynK44A-His_6, DynΔPHD-His_6, and Dyn2-His_6 were expressed and purified similarly.

GTPase assay and preparation of PS-liposomes. The Malachite Green GTPase assay of Dyn was performed as described previously [20]. A solution of PS in chloroform/methanol (95:5) (10 mg ml^{-1}, 40 μl) was evaporated to about 5 μl, resuspended in 1 ml of 30 mM Tris–HCl pH 7.4, and sonicated for 2 min on ice to afford a working solution of 400 μg ml^{-1}.

Surface plasmon resonance (SPR). SPR analyses were essentially performed as described previously [9]. SPR analyses were performed on a Biacore 3000 with a Sensorchip NTA (Biacore K.K.), using Buffer A (10 mM Tris–HCl, 10 mM NaCl, 2 mM $MgCl_2$, 0.05% Tween 80, pH 7.4) as the eluent (20 μl min^{-1}), at 25 °C. All four sensor sites were treated with 20 μl of 100 μM $NiSO_4$, then Dyn-His_6, DynK44A-His_6, and DynΔPHD-His_6 (50 μl, 10 μg ml^{-1}) were trapped *via* the His_6 tag on sites 1, 2, and 3, respectively. Site 4 was the blank control (Buffer A only). PS-liposomes (100 μl; 100 μg ml^{-1}) were injected simultaneously over all four sites, with or without 50 μM sertraline. Sensorgrams for specific interactions were obtained by subtracting the sensorgram for Buffer A from those for Dyn-His_6, DynK44A-His_6, and DynΔPHD-His_6 with BIA Evaluation Software.

Cell culture and fluorescence imaging. HeLa and SH-Sy5Y cells were cultured with 5% or 10% fetal bovine serum in DMEM, seeded onto collagen IV-coated coverslips, and transfected with pEGFP-Dyn1 or pEGFP-Dyn1K44A using Lipofectamin™2000. Adhering cells were treated with drugs (30 min, 37 °C) and with 1 μg ml^{-1} Alexa-CTB (30 min, on ice), then incubated with 5 μg ml^{-1} Alexa-Tf (5 min, 37 °C). The cells were washed with ice-cold 150 mM glycine (pH 2.0), fixed with 4% paraformaldehyde, rinsed with PBS, air-dried, mounted on Prolong Gold antifade reagent with DAPI, and observed with a Carl Zeiss LSM510 confocal microscope (63×/1.4 oil immersion objective). Excitation wavelengths of DAPI, GFP, Alexa-CTB, and Alexa-Tf were 405, 488, 543 and 633 nm, respectively.

Image analysis. Microscopic images were analyzed using ImageJ software version 1.42q (NIH, USA: http://rsb.info.nih.gov/ij/index.html) [24]. Outlines of cells were traced using the polygon selection tool, and fluorescence intensity was obtained for each cell.

Western blotting. Cells were lysed with SDS–PAGE sample buffer and lysates were subjected to 8% SDS–PAGE. Gels were blotted on PVDF membrane, which was incubated in 5% non-fat dried milk in TBST, then with anti-Dyn 1 (1:250), anti-Dyn 2 (1:250), anti-clathrin HC (1:250), or anti-β-actin (1:1000), followed by HRP-conjugated secondary antibody. Bands were evaluated with the ECL Western Blotting Detection System using a LAS-3000 (Fuji Photo Film).

Results

Sertraline preferentially and reversibly inhibits endocytosis of Tf

Chlorpromazine (CPZ) is well known, not only as an antipsychotic [19], but also as an inhibitor of clathrin-dependent endocytosis [25]. Citalopram is a SSRI, like sertraline [26]. Moreover, we previously reported that sertraline potently inhibited Dyn1 GTPase activity *in vitro* and that sertraline, CPZ and citalopram inhibited Dyn 1 GTPase with IC_{50} values of 7.3 ± 1.0, 47.2 ± 23.1 and >100 μM, respectively [20]. Therefore, we first compared the effects of sertraline, CPZ and citalopram on endocytosis of marker proteins in HeLa cells (Fig. 1). Sertraline and CPZ inhibited uptake of transferrin (Tf) and cholera toxin subunit B (CTB) into HeLa cells, while citalopram was ineffective (Fig. 1A and B). Interestingly, sertraline was strongly Tf-selective, while CPZ was less so (Fig. 1B). These results suggested that Dyn-dependent endocytosis of Tf was blocked *via* inhibition of Dyn GTPase by sertraline.

Time course analyses showed that most of the internalization of Tf was blocked within 5 min after addition of sertraline (Fig. 2A), while the Tf uptake was unaffected by addition of citalopram (Fig. 2B). At 20 min after washout of sertraline, endocytosis was found to have returned to control levels (Fig. 2C), suggesting that sertraline inhibition is rapidly reversible.

Sertraline also inhibits endocytosis of Tf in neuronal cells

We next examined the effect of sertraline on endocytosis in human neuroblastoma SH-Sy5Y cells. Internalization of Tf, but not CTB, was strongly inhibited by 20 μM sertraline in SH-Sy5Y cells (Fig. 3A). These results are consistent with the suppression of endocytosis observed in HeLa cells, as shown in Fig. 1A.

Sertraline inhibits Dyn 2 GTPase

Endogenous expression of Dyn isoforms in both HeLa and SH-Sy5Y cells was investigated by Western blotting in order to identify

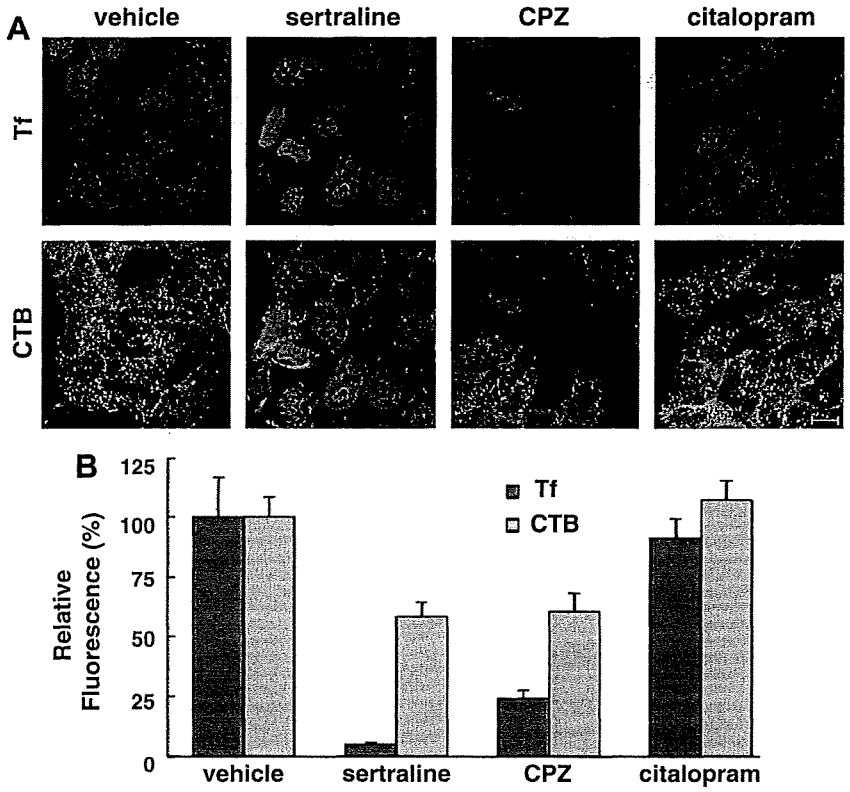

Fig. 1. Sertraline selectively blocks internalization of Tf in HeLa cells. Inhibition of endocytosis markers by sertraline in HeLa cells. (A) HeLa cells were treated for 30 min at 37 °C with vehicle, 20 μM sertraline, 20 μM CPZ or 20 μM citalopram. Upper panels, internalization of Tf (red); lower panels, internalization of CTB (orange). Nuclei were stained with DAPI (blue). Scale bar = 10 μm. (B) Relative fluorescence intensity. Internalized markers in the presence of vehicle, sertraline, CPZ and citalopram were measured by application of NIH-ImageJ (vehicle, $n = 13$; sertraline, $n = 19$; CPZ, $n = 13$; citalopram, $n = 25$). Relative fluorescence intensity was calculated in arbitrary units, based on the value for vehicle-treated cells as 100%. Red and orange columns show relative fluorescence intensity of Tf and CTB uptake, respectively. Error bars indicate SEM.

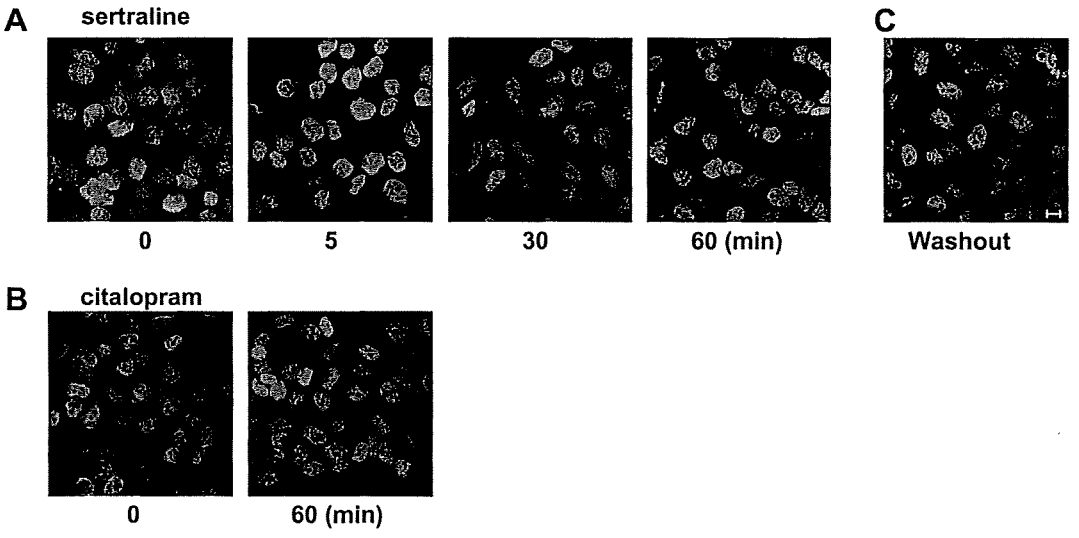

Fig. 2. The inhibitory effect of sertraline on Tf uptake in HeLa cells is reversible. Time course of inhibition of Tf (red) internalization by sertraline or citalopram. (A) Treatment with 20 μM sertraline for 0, 5, 30 or 60 min. (B) Treatment with 20 μM citalopram for 0 and 60 min. (C) Washout procedure: cells were treated with sertraline (30 min, 37 °C) and then with DMEM (20 min, 37 °C) to wash out sertraline. The washed cells were incubated with Alexa-Tf (5 min, 37 °C). Nuclei were stained with DAPI (blue). Scale bar = 10 μm.

the target of sertraline. We found that Dyn 1 was expressed in SH-Sy5Y cell extract, but was undetectable in HeLa cell extract (Fig. 3B). Dyn 2 and clathrin heavy chain (CHC) were expressed in both HeLa and SH-Sy5Y cells, as was β-actin, used as a loading control (Fig. 3B). Since sertraline blocked endocytosis equally effectively in HeLa and SH-Sy5Y cells (Figs. 1A and 3A), these results suggest that the effect of sertraline on endocytosis of Tf is mediated by inhibition of Dyn 2, not Dyn 1, at least in non-neuronal cells.

To investigate whether sertraline inhibits Dyn 2 GTPase activity, the Malachite Green GTPase assay was conducted. Sertraline inhibited Dyn2-His_6 with an IC_{50} value of 3.7 ± 1.3 μM as shown in Fig. 3C. In our previous study, the IC_{50} value of sertraline for Dyn