ity.[22] First, we examined whether each of the eight polymorphisms affected the mRNA expression or RNA editing level of *HTR2C* in the brain. We did not find significant effects of polymorphisms on the expression level of *HTR2C*, including –795C/T (Fig. 2) and Cys23Ser functional polymorphisms (data not shown). Similarly, we did not find significant effects of genetic variations on the RNA editing efficiency at site A or D (data not shown). We also confirmed that there were no significant effects of genetic variations on expression or RNA editing level using only control subjects (data not shown).

We then performed haplotype-based analysis using five polymorphisms in the promoter region (SNP #1–5 in Fig. 1). We conducted ANOVA using the three representative haplotype combinations (diplotypes; $A(GT)_{16}GCG/A(GT)_{16}GCG$ or $A(GT)_{16}GCG$/-, $A(GT)_{16}GCG/G(GT)_{13}ATC$, and $G(GT)_{13}ATC/G(GT)_{13}ATC$ or $G(GT)_{13}ATC$/-) as factor variables. However, we could not detect significant differences of expression or RNA editing efficiency across three diplotypes (Fig. 3). We then performed haplotype-based analysis using all detected polymorphisms (SNP #1–8 in Fig. 1) in the *HTR2C*. Although there were 12 diplotypes within 58 subjects, six diplotypes were omitted from analysis because of the small number of subjects ($n \leq 2$). Using the remaining six diplotypes as factor variables, we performed ANOVA again. However, we could not detect significant differences of expression or RNA editing efficiency across haplotypes (data

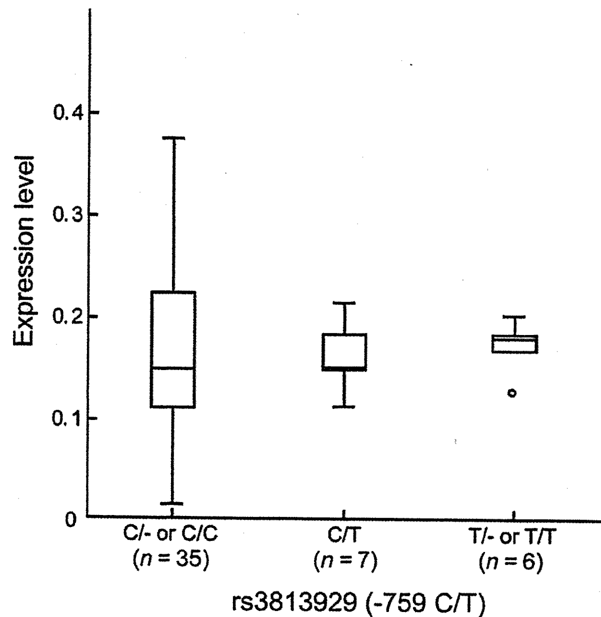

Figure 2. The rs3813929 (–795C/T) genotype and serotonin receptor 2C (*HTR2C*) mRNA expression level. In the box plot, top, bottom, and middle bars represent maximum, minimum, and median values, respectively. The top and bottom box represent third and first quartiles, respectively. Note that *HTR2C* is located on the X chromosome.

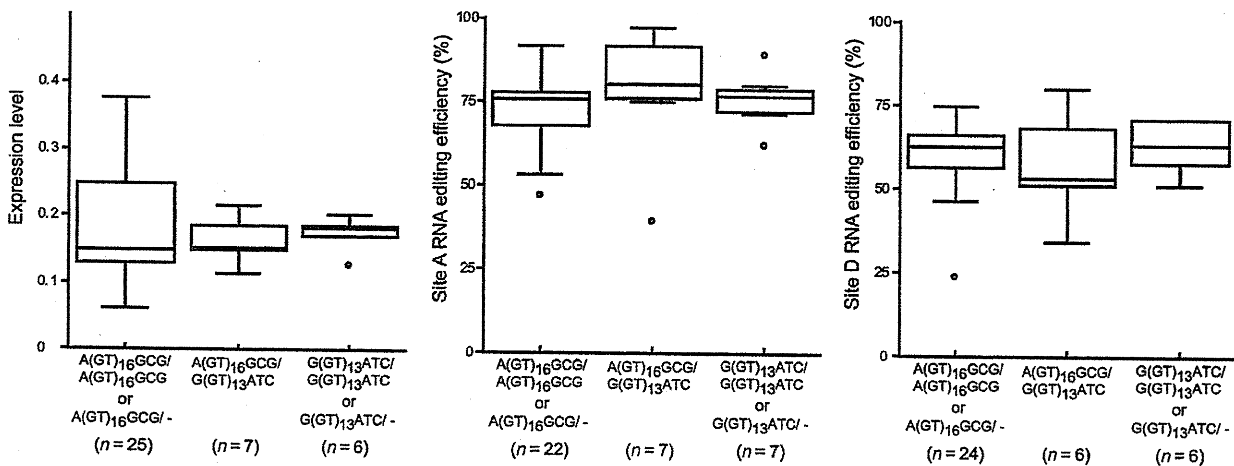

Figure 3. Promoter diplotype, mRNA expression level and RNA editing efficiency of the serotonin receptor 2C (*HTR2C*). Diplotypes were constructed using five promoter SNP (SNP #1–5 in Fig. 1).

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not shown). We also examined the relationship between expression level and RNA editing efficiency of *HTR2C* in the brain, which was not addressed in the previous studies, but we found no significant correlations between them ($R = 0.118$, $P = 0.450$ for site A, and $R = 0.163$, $P = 0.297$ for site D). Because *HTR2C* is located on chromosome X, we then performed the same analyses in male and female groups, respectively. However, we did not find significant relationship between these variables.

DISCUSSION

In the sample set used in this study, *HTR2C* expression showed a 1.55-fold decrease in BD and a 1.46-fold decrease in SZ.[17] We also reported a tendency toward an increase of RNA editing efficiency at site D in MD subjects and at site A in suicide victims.[18] Sequence analysis of *HTR2C* in the same cohort revealed that genetic variations of *HTR2C* did not affect the expression or RNA editing status in the brain. A similar result with regard to *HTR2C* expression in the brain was also reported by Castensson *et al.*[11] They compared the effect of haplotypes, which were similar to this study on the expression level of *HTR2C* in the brain, and they did not find significant differences either.[11]

In contrast, *in vitro* reporter assays revealed that promoter genotype or haplotype of *HTR2C* affected the transcriptional activity.[23–26] Among the studies, McCarthy *et al.* and Hill *et al.* used the haplotype constructs that were comparable to our study, for reporter assays.[25,26] They reported that haplotype $(GT)_{16}$GCG showed higher promoter activity than $(GT)_{13}$ATC. The cause of discrepancy might be due to the relatively small sample size ($n = 58$) in this study, which lacks the statistical power to detect the small effects. Indeed, power analysis revealed that, in our sample size, we could detect a 'large' effect size ($f = 0.4$)[27] at the power of 0.55 in ANOVA. However, it is also clear that the observed decreased expression of *HTR2C* in brains of patients cannot be fully explained by the genetic variations of *HTR2C*.

Therefore, other factors may be involved in the observed altered expression and RNA editing statuses of *HTR2C* in patients with mental disorders. Because we did not detect correlations between expression and the RNA editing level of *HTR2C*, independent factors may contribute to their altered statuses in the brain.

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BASIC NEUROSCIENCES, GENETICS AND IMMUNOLOGY - ORIGINAL ARTICLE

Effect of mood stabilizers on gene expression in lymphoblastoid cells

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Abstract Lithium and valproate are widely used as effective mood stabilizers for the treatment of bipolar disorder. To elucidate the common molecular effect of these drugs on non-neuronal cells, we studied the gene expression changes induced by these drugs. Lymphoblastoid cell cultures derived from lymphocytes harvested from three healthy subjects were incubated in medium containing therapeutic concentrations of lithium (0.75 mM) or valproate (100 μg ml^{-1}) for 7 days. Gene expression profiling was performed using an Affymetrix HGU95Av2 array containing approximately 12,000 probe sets. We identified 44 and 416 genes that were regulated by lithium and valproate, respectively. Most of the genes were not commonly affected by the two drugs. Among the 18 genes commonly altered by both drugs, vascular endothelial growth factor A (*VEGFA*), which is one of the *VEGF* gene isoforms, showed the largest downregulation. Our findings indicate that these two structurally dissimilar mood stabilizers, lithium, and valproate, alter *VEGFA* expression. *VEGFA* might be a useful biomarker of their effects on peripheral tissue.

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Keywords Lithium · Valproate · Lymphoblastoid cells · DNA microarray · Vascular endothelial growth factor A

Introduction

Bipolar disorder is a severe mental disorder characterized by recurrent episodes of mania and depression. Although the pathogenesis of bipolar disorder is unclear, mood stabilizers such as lithium and valproate can prevent its recurrence (Kato 2007). Lithium is the mainstay of treatment for bipolar disorder in many countries, both for acute mania and as prophylaxis for recurrent manic and depressive states (Geddes et al. 2004).

The molecular mechanism of their clinical actions as mood stabilizers has been extensively studied. Nonaka et al. (1998) and Chen et al. (1999c) demonstrated neuroprotective effects of lithium and valproate. These two mood stabilizers exert the same effects on neurons, such as growth cone enlargement (Williams et al. 2002) and enhanced adult neurogenesis (Chen et al. 2000; Hao et al. 2004). The effects may be mediated by depletion of inositol (Williams et al. 2002), upregulation of BCL-2 (Chen et al. 1999c), inhibition of glycogen synthase kinase 3b (Chen et al. 1999b; Klein and Melton 1996), altered glutamate receptor trafficking (Du et al. 2003), upregulation of BAG-1 (Zhou et al. 2005), inhibition of glucocorticoid receptor function (Basta-Kaim et al. 2004), upregulation of glutathione S-transferase (Cui et al. 2007), upregulation of brain-derived nerve growth factor (Yasuda et al. 2009), and activation of Notch signaling (Higashi et al. 2008). Inhibition of histone deacetylase (Leng et al. 2008) might also have a role in the neuroprotective effect of valproate.

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In most of these studies, neuronal cell cultures or neuronal cell lines were used, and very few studies examined the effect of these drugs on peripheral tissues or non-neuronal cell lines. The effects of these mood stabilizers on non-neuronal cells might also play a role in their clinical effects. Furthermore, a common molecular reaction between neurons and peripheral blood cells relevant to their clinical effects may be useful as a peripheral marker of the therapeutic response.

Although the usefulness of blood-derived samples such as lymphoblastoid cells (LCs) for studying mental disorders and pharmacogenetics is controversial (Choy et al. 2008; Matigian et al. 2008), several gene expression profiling studies of mental disorders have been performed using lymphocytes or LCs (Kakiuchi et al. 2003, 2008; Matigian et al. 2008; Sun et al. 2004; Vawter et al. 2004). Various phenotypes, such as abnormal Ca^{2+} levels (Emamghoreishi et al. 1997; Kato et al. 2003), altered endoplasmic reticulum stress response (Hayashi et al. 2009; Kakiuchi et al. 2003; So et al. 2007), and altered inositol levels (Belmaker et al. 2002), have been reported in LCs derived from patients with bipolar disorder.

Here we performed a comprehensive DNA microarray analysis of genes regulated by lithium and valproate in the LCs to identify their effects on peripheral tissue. Our findings indicated that lithium and valproate have distinctive as well as common effects on gene expression in LCs. Among the genes whose altered expression was common to lithium and valproate, vascular endothelial growth factor A (*VEGFA*) was the most downregulated. *VEGFA* might be a useful biomarker of their effects on peripheral tissue.

Materials and methods

Subjects

For DNA microarray and real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR), we used LCs derived from three unrelated healthy Japanese males (33, 42, and 49 years old). This study was approved by the Research Ethics Committee of RIKEN.

Lymphoblastoid cell culture

Lymphocytes from peripheral blood cells were transformed by Epstein–Barr virus using standard techniques as described previously (Kato et al. 2003). Briefly, lymphocytes were separated from peripheral blood and cultured with RPMI 1640 medium containing 20% fetal bovine serum (GIBCO, Carlsbad, CA, USA), penicillin and streptomycin (50 μg ml^{-1} each), and supernatant of the B95-8 cell culture infected by Epstein–Barr virus. The cells were passaged every week until the cells showed stable growth. Thereafter, the cells were passaged two or three times a week using similar medium, except for the addition of 10% fetal bovine serum.

To investigate the effects of the mood stabilizers, we used LCs derived from three unrelated healthy Japanese men. The cells were cultured in medium containing therapeutic concentrations of lithium (0.75 mM) and valproate (100 μg ml^{-1}) or drug-free control medium for 7 days.

DNA microarray procedure

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) and cleaned using an RNeasy column (Qiagen, Valencia, CA, USA). DNA microarray analysis was performed according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA) as described previously (Iwamoto et al. 2004). Briefly, 8–10 μg total RNA was used to synthesize cDNA by SuperScript II reverse transcriptase (Invitrogen), which was then used to generate biotinylated cRNA. cRNA was fragmented and first applied to the TestChip (Affymetrix) to assess the sample quality, and then applied to the HU95Av2 chip (Affymetrix), which contained approximately 12,000 probe sets. The hybridization signal on the chip was scanned using a scanner (HP GeneArray scanner, Hewlett-Packard, Palo Alto, CA, USA), and processed with microarray suite 5 (MAS5, Affymetrix).

DNA microarray data analysis

DNA microarray raw data were imported into GeneSpring GX10 software (Agilent Technologies, Santa Clara, CA, USA). Data from each array were then normalized by dividing each value by the median whole gene expression value. We then conducted a principal component analysis (PCA) to visualize the relationship among experiments using the expression values of all probe sets on the array. To examine quantitative expression changes in response to drug treatment, we used probe sets whose flag statuses were present or marginal in at least five of nine samples for statistical analysis. Of approximately 12,000 probe sets, 5,656 probe sets passed this filtering process. Genes that were differentially expressed compared with control samples were identified using one-way analysis of variance [ANOVA ($df = 2$, lithium-treated, valproate-treated, and control samples)] followed by post hoc Tukey's test. A P value of less than 0.05 was considered significant. Genes with significantly different levels of expression between drug-treated and control samples were selected. Gene ontology analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) software (Dennis et al. 2003; Huang da et al. 2009). The significantly enriched terms were identified using Bonferroni's correction for multiple comparisons with a cutoff of $P < 0.05$.

Real-time quantitative RT-PCR

After DNase I treatment, 5 μg total RNA was used for cDNA synthesis by oligo(dT) 12–18 primer and SuperScript II reverse transcriptase (Invitrogen). Real-time quantitative RT-PCR using SYBER/GREEN I dye (Applied Biosystems, Foster City, CA, USA) was performed with ABI PRISM 7900HT (Applied Biosystems). After denaturation at 95°C for 10 min, the PCR conditions were 95°C for 45 s and 60°C for 45 s for 40 cycles. The comparative C_t method was used to quantify transcripts according to the manufacturer's protocol (User Bulletin #2, Applied Biosystems). Each sample was quantified in triplicate. Amplification of single products was confirmed by monitoring the dissociation curve and by agarose gel electrophoresis. We used the expression level of cofilin 1 as an internal control, as validated previously (Iwamoto et al. 2004). Primer pairs for cofilin 1 were reported previously (Iwamoto et al. 2004). Primer pairs used for qRT-PCR were as follows: *VEGFA*, 5′-TACTGCCATCCAATCGAGACC-3′ and 5′-GGTTTG ATCCGCATAATCTGC-3′. Two-tailed paired *t*-tests were used for the statistical analysis of the results of the control and lithium or valproate-treated samples.

Results

LCs established from three unrelated male subjects were cultured with therapeutic concentrations of lithium or valproate. After 7 days culture, total RNA was extracted from the cells and used for DNA microarray analysis. After data normalization, we first performed a PCA to visualize the relationship among the lithium- or valproate-treated samples and control samples (Fig. 1). The lithium-treated samples differed little from their respective control samples. On the other hand, the valproate-treated samples were quite different from the control samples. These results suggest the slight and drastic effects of lithium and valproate, respectively, on global gene expression in LCs in our experimental condition.

To identify differentially expressed probe sets, we performed an ANOVA followed by Tukey's test. Lithium induced altered expression in 44 probe sets, of which 17 were downregulated and 27 were upregulated (see Supplementary Table 1). On the other hand, valproate induced altered expression in 416 probe sets, of which 222 were downregulated and 194 were upregulated (see Supplementary Table 2). The ten most downregulated and upregulated probe sets affected by lithium and valproate treatment are listed in Tables 1 and 2, respectively, as representatives. Among the identified probe sets, the expression of 18 probe sets was altered by both lithium and valproate. Alterations of all of these 18 probe sets were in the same direction (Table 3).

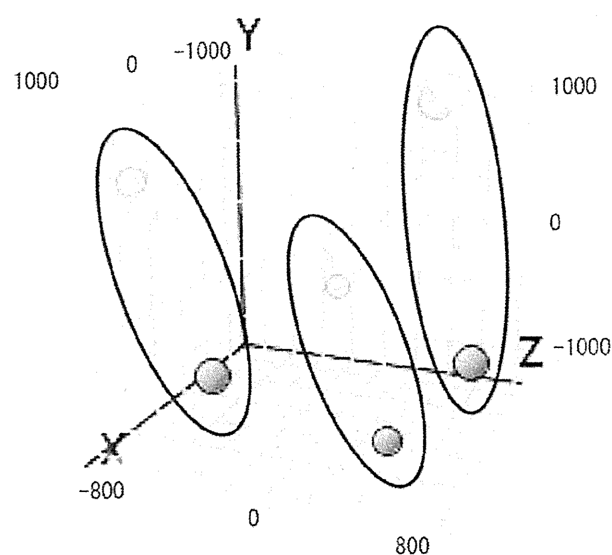

Fig. 1 Results of the PCA analysis. *X*, *Y*, and *Z* axes show the first, second, and third components, respectively. *White*, *gray*, and *black spheres* show control, lithium, and valproate-treated samples, respectively. Each ellipse surrounds the data of the same individual

A gene ontology analysis was then performed using the DAVID software (Table 4). The differential gene expression induced by valproate was significantly enriched in metabolic process-related and cellular process-related terms in the biologic process category, and nucleic acid-binding and protein-binding terms in the molecular function category. In the cellular component category, genes seemed to be enriched in every part of the cell, including the nucleus, cytoplasm, and organelles. We also performed a gene ontology analysis of the differential gene expression induced by lithium and that induced by both drugs. No significant enrichment was observed, however, after multiple testing corrections, possibly due to the small number of genes used for analysis (data not shown).

We also analyzed the expression level of *VEGFA*, which was the gene most downregulated by both lithium and valproate, using real-time quantitative RT-PCR (Fig. 2). We confirmed significant downregulation of *VEGFA* in valproate-treated LCs ($P = 0.02$). We also observed downregulation of *VEGFA* in lithium-treated LCs, but this result was not significant ($P = 0.21$).

Discussion

Both lithium and valproate are widely used in the treatment of bipolar disorder as mood stabilizers. These two structurally unrelated drugs have similar clinical efficacy for

Table 1 The ten genes most downregulated and upregulated by lithium

Probe ID	Accession	Fold change	Gene symbol	Gene title
2067_f_at	D44466	−1.86	BAX	BCL2-associated X protein
2065_s_at	U67092	−1.70	BAX	BCL2-associated X protein
37632_s_at	U19599	−1.62	ZRF1	Zuotin related factor 1
38837_at	L22473	−1.59	TXNDC13	Thioredoxin domain containing 13
450_g_at	L22475	−1.57	CGRRF1	Cell growth regulator with ring finger domain 1
1997_s_at	X78283	−1.57	BAX	BCL2-associated X protein
36639_at	AC002985	−1.51	ADSL	Adenylosuccinate lyase
36101_s_at	AF060798	−1.45	VEGFA	Vascular endothelial growth factor A
41003_at	X79201	−1.37	PFDN4	Prefoldin subunit 4
34417_at	D63390	−1.35	DPY19L2P2	dpy-19-like 2 pseudogene 2 (*C. elegans*)
32445_at	AF057160	2.03	PAFAH1B2	Platelet-activating factor acetylhydrolase, isoform Ib, beta subunit 30 kDa
31872_at	X98260	1.87	SS18	Synovial sarcoma translocation, chromosome 18
38365_at	X80754	1.72	PEX1	Peroxisome biogenesis factor 1
41601_at	D25547	1.68	–	Transcribed locus
37837_at	AB020670	1.67	ADNP2	ADNP homeobox 2
940_g_at	D87449	1.64	NF1	Neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)
37888_at	AB007858	1.57	SLC35D1	Solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter), member D1
31804_f_at	Y10055	1.50	SULT1A1	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1
31868_at	AF026086	1.47	STK16	Serine/threonine kinase 16
33731_at	AA552140	1.47	SLC7A7	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 7

Among the 27 probe sets identified, the top ten downregulated and upregulated probe sets are listed as representative

bipolar disorder, and thus we searched for a common molecular effect of these drugs. Although these drugs share clinical efficacy for classical mania, valproate has specific efficacy for irritable or dysphoric mania (Swann et al. 2002). Therefore, lithium and valproate may have common and drug-specific molecular effects. At the gene expression level in the LCs, the effect of lithium was modest compared with that of valproate. These two drugs showed distinctive gene expression changes with altered expression of only a very small number of common genes.

Gene expression changes induced by lithium

In our experiments, the effect of lithium on gene expression was relatively small. In recent studies, using mouse brain (Du et al. 2003) or human neuronal cell lines treated with lithium (Seelan et al. 2008), altered expression of more genes was reported. These differences may reflect the differences in the tissues or treatment methods used. The expression of three different probe sets for BCL-2 associated X protein (*BAX*) was consistently downregulated by lithium (Table 1). BAX is a BCL-2 family protein that promotes apoptosis by enhancing cytochrome c release (Graham et al. 2000). Several studies have reported that lithium upregulates anti-apoptotic BCL-2 and downregulates pro-apoptotic BAX (Chen and Chuang 1999; Ghribi et al. 2002; Somervaille et al. 2001). Our findings are consistent with these findings in the brain and neuronal cells.

On the other hand, the gene most upregulated by lithium was platelet-activating factor acetylhydrolase, isoform 1b, beta subunit (*PAFAH1B2*), which encodes one of the catalytic subunits of the PAFAH1B complex, alpha2. The PAFAH1B alpha complex is an enzyme composed of alpha2, alpha1 encoded by *PAFAH1B3*, and Lis1 encoded by *PAFAH1B1* (Hattori et al. 1993). Among them, *PAFAH1B3* has been identified as one of eight genes that show specific alterations in the postmortem brains of bipolar individuals (Nakatani et al. 2006). These enzymes interact biochemically and genetically with the Reelin (*RELN*) pathway, and RELN and Lis activities are important for neuronal migration and cellular layer formation in the brain (Assadi et al. 2003; Hirotsune et al. 1998). The Pafah1b alpha2 subunit encoded by the *Pafah1b2* gene reportedly does not affect cortical layer formation, but is critically involved in the development of hydrocephalus in double mutant mice defective in Reelin and Lis signaling (Assadi et al. 2008). Considering the possible role of the

Table 2 The ten genes most downregulated and upregulated by valproate

Probe ID	Accession	Fold change	Gene symbol	Gene title
39827_at	AA522530	−2.72	DDIT4	DNA-damage-inducible transcript 4
36398_at	W28729	−2.71	–	Human retina cDNA
36101_s_at	M63978	−2.52	VEGFA	Vascular endothelial growth factor A
37326_at	U93305	−2.51	PLP2	Proteolipid protein 2 (colonic epithelium-enriched)
35530_f_at	X92997	−2.45	–	Immunoglobulin lambda locus
282_at	L16782	−2.45	MPHOSPH1	M-phase phosphoprotein 1
40790_at	AB004066	−2.28	BHLHB2	Basic helix-loop-helix domain containing, class B, 2
300_f_at[a]	–	−2.23	–	–
40329_at	AL031228	−2.23	SLC39A7	Solute carrier family 39 (zinc transporter), member 7
38010_at	AF002697	−2.22	BNIP3	BCL2/adenovirus E1B 19 kDa interacting protein 3
35778_at	AB011103	6.20	KIF5C	Kinesin family member 5C
668_s_at	L22524	5.20	MMP7	Matrix metallopeptidase 7 (matrilysin, uterine)
36874_at	M26004	4.34	CR2	Complement component (3d/Epstein–Barr virus) receptor 2
1575_at	M14758	3.85	ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1
40862_i_at	X15334	3.63	CKB	Creatine kinase, brain
1358_s_at	U22970	3.40	IFI6	Interferon, alpha-inducible protein 6
915_at	M24594	3.09	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
32814_at	M24594	3.05	IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
38584_at	AF026939	2.89	IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
33232_at	AI017574	2.85	CRIP1	Cysteine-rich protein 1 (intestinal)/hypothetical protein LOC100127933

Among the 416 probe sets identified, the top 10 downregulated and upregulated probe sets are listed as representative

[a] 300_f_at was not annotated

PAFAH1B2 gene in brain development, an increase in *PAFAH1B2* may contribute to the mechanism of action of mood stabilizers, especially lithium.

Gene expression changes induced by valproate

Valproate is characterized as a histone deacetylase inhibitor (Phiel et al. 2001). Histone deacetylases control the transcription of a large number of genes by altering chromosome dynamics. In the present study, the PCA revealed more extensive gene expression alterations induced by valproate than by lithium. Statistical analysis also revealed that valproate affected a larger number of differentially expressed probe sets than lithium. Gene ontology analysis revealed that valproate affected the expression of genes with wide-ranging biologic functions. Consistent with previous studies in the brain (Bosetti et al. 2005; Chetcuti et al. 2006), we determined that valproate also affects multiple signaling pathways in LCs. BCL-2/adenovirus E1B 19 kDa interacting protein (*BNIP3*) was one of the ten most downregulated genes by valproate (Table 2). *BNIP3*, which is a BCL-2 family member, is upregulated during stress such as hypoxia and induces cell death by inducing mitochondrial dysfunction (Kim et al. 2002; Vande Velde et al. 2000). Downregulation of *BNIP3* may also contribute to the cytoprotective effect of valproate.

Altered gene expression commonly induced by lithium and valproate

Eighteen probe sets were identified as differentially expressed genes common to lithium and valproate. *VEGFA*, strongly associated with angiogenesis in the *VEGF* gene family (Ferrara 2002), was the gene most downregulated by both lithium and valproate. The expression level of *VEGFA* was significantly decreased by valproate, and tended to be decreased by lithium in real time RT-PCR.

VEGF was initially identified as a critical regulator of angiogenesis and a potent inducer of vascular permeability in many types of tissues, including the blood brain barrier (Ferrara et al. 2003; Gora-Kupilas and Josko 2005; Rigau et al. 2007). *VEGF* also has neurogenic and neuroprotective effects (Jin et al. 2002; Schanzer et al. 2004; Storkebaum et al. 2004). Recent studies indicate that *VEGF* may be involved in the molecular mechanisms of the pathogenesis and response to treatment for depression. In animal studies, *VEGF* is induced by antidepressant drugs (Warner-Schmidt and Duman 2007) and electroconvulsive shock

Table 3 The list of common differentially expressed genes by lithium and valproate

Probe ID	Accession	Fold change		Gene symbol	Gene title
		Lithium	Valproate		
36101_s_at	D44466	−1.45	−2.52	VEGFA	Vascular endothelial growth factor A
41003_at	X79201	−1.37	−1.39	PFDN4	Prefoldin subunit 4
34417_at	AL049437	−1.35	−1.63	DPY19L2P2	dpy-19-like 2 pseudogene 2 (*C. elegans*)
37737_at	H93123	−1.33	−1.46	PCMT1	Protein-L-isoaspartate (D-aspartate) *O*-methyltransferase
41595_at	AF074382	−1.22	−1.25	KIAA0947	KIAA0947 protein
38073_at	M63978	−1.15	−1.16	RNMT	RNA (guanine-7-) methyltransferase
31872_at	D25547	1.87	1.83	SS18	Synovial sarcoma translocation, chromosome 18
940_g_at	D87449	1.64	1.62	NF1	Neurofibromin 1 (neurofibromatosis, von Recklinghausen disease, Watson disease)
37888_at	AB007858	1.57	1.64	SLC35D1	Solute carrier family 35 (UDP-glucuronic acid/UDP-*N*-acetylgalactosamine dual transporter), member D1
38706_at	Y10055	1.47	1.49	E2F4	E2F transcription factor 4, p107/p130-binding
381_s_at	AA552140	1.45	1.90	PIK3CD	Phosphoinositide-3-kinase, catalytic, delta polypeptide
38945_at	X78710	1.34	1.66	MTF1	Metal-regulatory transcription factor 1
39434_at	AB011164	1.33	1.45	FAM21A/B/C/D	Family with sequence similarity 21, member A/B/C/D
35783_at	AF071504	1.30	1.57	VAMP3	Vesicle-associated membrane protein 3 (cellubrevin)
41047_at	U41816	1.26	1.24	C9orf16	Chromosome 9 open reading frame 16
36004_at	AI885170	1.22	1.29	IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma
39858_s_at	AB023164	1.21	1.37	STX11	Syntaxin 11
1314_at	D12625	1.20	1.32	PSMD1	Proteasome (prosome, macropain) 26S subunit, non-ATPase, 1

(Altar et al. 2004; Newton et al. 2003; Segi-Nishida et al. 2008). Clinical studies demonstrated a higher expression level of *VEGF* mRNA and a decrease in these levels after treatment with antidepressants was observed in the peripheral leukocytes of depressed patients (Iga et al. 2007), but other studies reported conflicting results (Dome et al. 2009; Ventriglia et al. 2009).

Lithium increases myocardial levels of *VEGF* following ischemic insult (Kaga et al. 2006), promotes *VEGF* expression in brain endothelium and astrocytes (Guo et al. 2009), and prevents *VEGF* reduction in immature neurons of the hippocampus after chronic mild stress in rats (Silva et al. 2007). *VEGF* is decreased in a human neuronal cell line continuously maintained on therapeutic levels of lithium (Seelan et al. 2008). In addition, valproate promotes tumor growth arrest by downregulating *VEGF* (Gao et al. 2007; Zgouras et al. 2004).

In our experiments, both lithium and valproate decreased the gene expression of *VEGFA*. As described above, the direction of *VEGF* expression changes differed across studies. The discrepancy might be due to differences in the experimental methodology, such as in vivo or in vitro, the type of tissues, and treatment method. We used LCs derived from peripheral blood lymphocytes, and therefore, our findings suggest that the mechanisms of action of these mood stabilizers may be mediated in part by their effects on *VEGF* in non-neuronal cells. Valproate inhibits angiogenesis in endothelial cells, thereby protecting endothelial cells from stress-induced apoptosis (Michaelis et al. 2006). It is possible that the common antiangiogenic effect of lithium and valproate mediated by VEGF contributes to the neuroprotective effects in bipolar disorder.

Limitations

Although we used LCs in our experiment, the effect of mood stabilizers on non-neuronal cells might differ among various tissues. In addition, because the difference in drug sensitivity between non-neuronal cell and neuronal cell is suggested (Chen et al. 1999a; Gilad and Gilad 2007), we should examine lower and higher (toxic) dose of these drugs to explain whether the results are based on the treatment effects or toxic effects. The effects of mood stabilizers on other non-neuronal cells should also be studied. In our experiment, we used LCs rather than fresh lymphocytes because LCs are easily handled and cellular heterogeneity can be minimized. Whether this effect is also detected in non-transformed peripheral lymphocytes and can be used as a peripheral biomarker of the effects of mood stabilizers requires further study. The microarray data results did not cover the entire genome because the number of probe sets contained in the HU95Av2 chip is smaller than that of newer chip versions. Although we

Table 4 The result of gene ontology analysis of valproate

Term	Count	Fold enrichment	*P* value	Bonferroni
Biological process				
GO:0044237~cellular metabolic process	231	1.27	5.28E−08	2.78E−04
GO:0043170~macromolecule metabolic process	208	1.31	7.09E−08	3.73E−04
GO:0008152~metabolic process	249	1.23	8.62E−08	4.53E−04
GO:0044238~primary metabolic process	229	1.25	2.38E−07	1.25E−03
GO:0043283~biopolymer metabolic process	163	1.38	4.28E−07	2.25E−03
GO:0007049~cell cycle	45	2.28	4.40E−07	2.31E−03
GO:0009987~cellular process	315	1.10	1.64E−06	8.57E−03
GO:0006139~nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	128	1.43	3.31E−06	1.72E−02
GO:0016043~cellular component organization and biogenesis	94	1.56	4.43E−06	2.30E−02
GO:0051649~establishment of cellular localization	42	2.16	4.60E−06	2.39E−02
GO:0051641~cellular localization	42	2.10	9.04E−06	4.64E−02
Cellular component				
GO:0005622~intracellular	313	1.38	2.86E−28	2.48E−25
GO:0044424~intracellular part	306	1.43	4.32E−30	3.75E−27
GO:0043229~intracellular organelle	264	1.48	6.19E−22	5.37E−19
GO:0043226~organelle	264	1.48	6.76E−22	5.87E−19
GO:0043231~intracellular membrane-bound organelle	241	1.55	2.11E−21	1.83E−18
GO:0043227~membrane-bound organelle	241	1.55	2.25E−21	1.96E−18
GO:0005737~cytoplasm	216	1.61	1.49E−19	1.29E−16
GO:0044444~cytoplasmic part	133	1.66	1.41E−10	1.22E−07
GO:0044446~intracellular organelle part	126	1.67	4.13E−10	3.59E−07
GO:0044422~organelle part	126	1.66	5.10E−10	4.42E−07
GO:0005634~nucleus	152	1.51	2.92E−09	2.53E−06
GO:0032991~macromolecular complex	97	1.72	2.95E−08	2.56E−05
GO:0044428~nuclear part	48	2.10	1.77E−06	1.54E−03
GO:0043234~protein complex	77	1.69	3.30E−06	2.86E−03
GO:0005739~mitochondrion	43	2.07	9.83E−06	8.50E−03
GO:0044464~cell part	338	1.04	5.37E−05	4.56E−02
GO:0005623~cell	338	1.04	5.47E−05	4.64E−02
Molecular function				
GO:0003676~nucleic acid binding	119	1.54	2.51E−07	7.22E−04
GO:0005515~protein binding	233	1.54	6.13E−19	1.76E−15
GO:0005488~binding	313	1.15	4.54E−08	1.31E−04
GO:0003677~DNA binding	82	1.60	1.19E−05	3.37E−02

rigorously analyzed the microarray data, the results may contain false positives, and thus need to be confirmed by independent technologies.

Conclusion

In summary, we performed a comprehensive DNA microarray analysis to examine gene expression profiles in LCs exposed to lithium or valproate. Lithium and valproate had distinctive effects on gene expression in the LCs, suggesting that different molecular mechanisms are involved in the mechanism of action of these two mood stabilizers. Among the genes commonly altered by lithium and valproate, *VEGFA* was the gene most downregulated by both drugs. The mechanisms of action of these mood stabilizers may be mediated in part by their effects on *VEGFA* in non-neuronal cells, and *VEGFA* may therefore be useful as a peripheral marker of the effects of mood stabilizers.

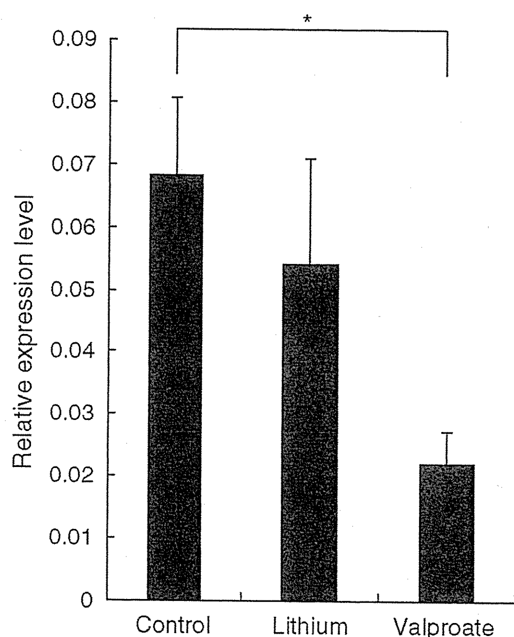

Fig. 2 The results of real-time quantitative RT-PCR. The expression level (mean ± SD) of *VEGFA* in control, lithium-treated, and valproate-treated LCs. $*P < 0.05$

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Anti-Inflammatory Effects of Antidepressants: Possibilities for Preventives Against Alzheimer's Disease

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Abstract: Increasing evidence of pro-inflammatory mediator expression in major depressions indicate that inflammatory changes may play a role. If this is true, the efficacy of antidepressants may be partially attributable to suppression of inflammation. Various types of antidepressants can suppress serum and plasma levels of pro-inflammatory mediators in patients with major depression. Therefore they can inhibit the production of pro-inflammatory mediators by immune cells. These include glial cells, which are the main sources and targets of cytokines in the brain. This review summarizes the evidence showing that antidepressants have an anti-inflammatory potential. The putative mechanisms are also discussed. Because of the anti-inflammatory effects of antidepressants, they might also act as preventives for neurodegenerative dementias including Alzheimer's disease, where the pathogenesis involves chronic inflammation associated with activated microglia.

Keywords: Antidepressants, major depression, Alzheimer's disease, inflammation, cytokines, microglia.

INTRODUCTION

The history of antidepressant drug development has been unique and fortuitous. The monoamine oxidase inhibitor iproniazid and the tricyclic antidepressant (TCA) imipramine were originally developed as a tuberculosis remedy and as an antihistamine, respectively [1]. These drugs were serendipitously found to have an antidepressant effect in the 1950s, and soon thereafter were shown to increase synaptic levels of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) [1]. Currently, it has been shown that antidepressants modulate not only the monoamine neurotransmitter system but also the inflammatory system.

The association between inflammation and major depression has been supported by the well-known observation that pro-inflammatory cytokines such as interferon (IFN)-α, which is used to treat patients with hepatitis C, and interleukin (IL)-2, which is used to treat patients with certain cancers, frequently induce depressive symptoms as side effects. In addition, depression is often found in inflammatory diseases such as multiple sclerosis, allergies of different types, and rheumatoid arthritis, in which pro-inflammatory cytokines are over-expressed [2]. Animal studies also support this idea. Chronic administration of the endotoxin lipopolysaccharide (LPS) or pro-inflammatory cytokines into rats has been shown to induce symptoms similar to depression. These symptoms are referred to as sickness behavior, which includes appetite loss, suppressed sexual behavior and apathy [3, 4].

It can be hypothesized that if inflammation plays a causative role in the pathogenesis of major depression, antidepressants may partially act by suppressing such inflammation. The first evidence indicating that antidepressants have anti-inflammatory effects appeared four decades ago. Martelli *et al.* (1967) showed that administration of TCAs inhibited chemically induced edema in the standard rat paw assay [5]. Ten years later, Horrobin and colleagues reported that the TCA clomipramine was a powerful antagonist of prostaglandin (PG) E_2 [6] and then proposed that diverse antidepressants are inhibitors of PG synthesis [7]. In fact, a recent *in vitro* study revealed that the selective serotonin reuptake inhibitor (SSRI) paroxetine attenuated cyclooxygenase (COX)-2 expression in human T cells stimulated with phytohemagglutinin (PHA) [8]. Furthermore, experimental evidence is accumulating that various types of antidepressants exert anti-inflammatory effects by decreasing pro-inflammatory cytokine levels or increasing anti-inflammatory cytokine levels.

This review focuses on the influence of antidepressants on inflammatory mediator levels, particularly serum and plasma cytokine levels, in depressed patients. It also focuses on glial production of those mediators *in vitro* since glial cells are the major immune cells responsible for inflammation in the brain. We also discuss possible mechanisms of the anti-inflammatory action of antidepressants and the potential of antidepressants to act as preventives against Alzheimer's disease (AD).

EVIDENCE FOR INFLAMMATION ASSOCIATED WITH MAJOR DEPRESSION

It has been reported that the levels of acute phase proteins such as C-reactive proteins (CRP), α2-macroglobulin, α1-acid glycoprotein, complement C4 and haptoglobin are

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upregulated in major depression [9-14]. The levels of PGE_2 and thromboxane B_2 are also reported to be elevated in depressed patients [15-17]. Moreover, major depression is accompanied by increased levels of pro-inflammatory cytokines such as IL-1β, IL-6, IFN-γ and tumor necrosis factor (TNF)-α [18-23] whereas the anti-inflammatory cytokine transforming growth factor (TGF)-β1 has been shown to be decreased [24].

In contrast to many studies on serum and plasma levels, there have been few on cerebrospinal fluid (CSF) levels. Only IL-1β has been shown to be increased in major depression [25] while IL-6 was decreased [25] or not changed [26] and TNF-α was not changed [25].

It is uncertain whether inflammation is a cause or a result of major depression. In addition, it must be noted that not all studies have found such an association [27, 28]. Nevertheless, inflammation certainly appears to be a factor in at least some cases of major depression. Indeed, Müller *et al.* (2006) have recently shown interesting data that depressed patients treated for 6 weeks with the serotonin-noradrenaline reuptake inhibitor (SNRI) reboxetine plus the COX 2 inhibitor celecoxib showed significantly greater improvement in scores on the Hamilton Depression Scale compared to the reboxetine-alone group [29].

EFFECT OF ANTIDEPRESSANTS ON INFLAMMATORY MEDIATOR LEVELS IN PATIENTS WITH MAJOR DEPRESSION

Several groups have studied serum or plasma levels of various cytokines and their receptors in patients with major depression before and after antidepressant pharmacotherapy (Table **1**, for a summery of studies before 2000 see [30]). Tuglu *et al.* (2003) showed that administration of SSRIs for 6 weeks decreased serum levels of TNF-α and CRP [23]. Basterzi *et al.* (2005) showed that similar SSRI treatment diminished serum IL-6 levels [31]. In keeping with such an anti-inflammatory effect, Myint *et al.* (2005) reported that 8-weeks of antidepressant treatment increased plasma TGF-β1 levels [24]. Interestingly, it has been shown that plasma levels of TNF-α and IL-6 in patients with SSRI-resistant depression are significantly higher than those in healthy controls [32]. However, Kubera *et al.* (2000) demonstrated that a 6-week antidepressant treatment which elicited successful clinical remissions did not change significantly the serum levels of IL-6, IL-10 and IL-1 receptor antagonist [33]. Two studies even described increases in the plasma TNF-α levels following antidepressant treatment. Kraus *et al.* (2002) reported that a 4-week treatment with a tetracyclic antidepressant (i.e., mirtazapine) increased the plasma levels of TNF-α and soluble TNF-α receptors significantly while a similar treatment with the SNRI venlafaxine did not influence those levels [34]. Kagaya *et al.* (2001) showed that plasma TNF-α level was increased after 1-month pharmacotherapy consisting mainly of clomipramine. They also examined the plasma levels of IL-1β and IL-6. Those levels after treatment were lower than before treatment, but not significantly [35].

Taken together, the effect of antidepressants on serum and plasma levels of inflammatory cytokines in depressed patients is still controversial. Such an inconsistency may stem from the difference in methodology employed and the limitation due to the small numbers tested in these clinical studies (e.g., $n<30$ in each study). In addition, Kennis and Maes (2002) pointed out the technical difficulty in detecting serum and plasma levels of cytokines since circulating cytokine levels are very low in human subjects [30]. Therefore, early studies on the cytokine concentrations before and after antidepressant treatment often employed *ex vivo* methods. Specifically, cytokine levels in the supernatants of cultured whole blood or cultured peripheral blood mononuclear cells (PBMCs) from depressed patients were measured by enzyme-linked immunosorbent assay (ELISA). In both cases, cytokine production was induced by stimulation with LPS and/or mitogens such as PHA and concanavalin A. Such *ex vivo* studies have shown inconsistent results on protein levels (for review see [30]).

Recently, Tsao *et al.* (2006) examined mRNA expression of inflammatory cytokines in non-stimulated PBMCs from depressed patients before and after 3-month SSRI (i.e., fluoxetine) treatment by using reverse transcriptase-polymerase chain reaction (RT-PCR) assy. They found that such pharmacotherapy significantly diminished the mRNA expression of IFN-γ. The mRNA expressions of IL-1β and TNF-α were also inhibited, but not significantly [36].

EFFECT OF ANTIDEPRESSANTS ON GLIAL PRODUCTION OF INFLAMMATORY MEDIATORS *IN VITRO*

With regard to *in vitro* studies, various types of antidepressants have anti-inflammatory effects in terms of cytokine production by immune cells. Early studies focused on the effects of antidepressants on cytokine production by cultured PBMCs or cultured whole blood from healthy subjects or depressed patients. They demonstrated that *in vitro* treatment with various types of antidepressants decreased the production of pro-inflammatory cytokines including IFN-γ while increasing the production of such anti-inflammatory cytokines as IL-10 (for reviews see [30, 37]). Moreover, a TCA (amitriptyline) and a SSRI (fluoxetine) were shown to attenuate the production of pro-inflammatory cytokine-induced PGE_2 and nitric oxide (NO) by cultured human synovial cells [38].

Increasing evidence strongly suggests that changes in cytokine levels outside the brain cause changes in cytokine expression and activity in the brain, and *vice versa* [39]. In other words, the central and peripheral cytokine compartments are integrated but differently regulated [40]. In the brain, microglia and astrocytes are the major cell types that participate in the inflammatory system both as sources and targets of cytokines. This fact suggests that these glial cells may represent overlooked targets in the etiology of major depression. Several studies have recently investigated the effects of antidepressants on the glial production of inflammatory mediators *in vitro* (Table **2**).

Obuchowicz *et al.* (2006) examined the effects of amitriptyline and its metabolite nortriptyline on the production of IL-1β and TNF-α by rat microglial and mixed glial (i.e., microglia plus astrocytes) cultures stimulated with LPS, using both ELISA and quantitative RT-PCR. They found

Table 1. Summary of Studies on Serum/Plasma Levels of Inflammatory Mediators in Depressed Patients Before and After Antidepressant Therapy

Study	n	Antidepressants	Target Studied	Result
Tuglu *et al.* (2003)	26	SSRIs (mostly Sertraline/Citalopram)	TNF-α	Decrease
			CRP	Decrease
Basterzi *et al.* (2005)	23	SSRIs (not specified)	IL-6	Decrease
Myint *et al.* (2005)	10	Various types (mostly Paroxetine/Fluoxetine)	TGF-β1	Increase
Kubera *et al.* (2000)	9	Not specified	IL-6	No change
			IL-10	No change
			IL-1RA	No change
Kraus *et al.* (2002)	9	SNRI (Venlafaxine)	TNF-α	No change
			sTNF-Rs	No change
	11	Tetracyclic (Mirtazapine)	TNF-α	Increase
			sTNF-Rs	Increase
Kagaya *et al.* (2001)	12	Mostly TCA (Clomipramine)	TNF-α	Increase
			IL-1β	No change
			IL-6	No change

IL-1RA, IL-1 receptor antagonist
sTNF-Rs, soluble TNF receptors

Table 2. Summary of Studies that Examined the Effect of Antidepressants on Glial Production of Inflammatory Mediators *In Vitro*

Study	Cell Used	Antidepressants	Target Studied	Result
Obuchowicz *et al.* (2006)	Rat microglia Rat mixed glia	TCAs (Amitriptyline/Nortriptyline)	IL-1β	Decrease
			TNF-α	Decrease
			IL-1β mRNA	No change
			TNF-α mRNA	No change
Hashioka *et al.* (2007)	Mouse microglia (6-3)	TCA (Imipramine)	IL-6	Decrease
			NO	Decrease
		SSRI (Fluvoxamine)	IL-6	Decrease
			NO	Decrease
		SNRI (Reboxetine)	IL-6	Decrease
			NO	Decrease
		LiCl	IL-6	Increase
			NO	Decrease
Vollmar *et al.* (2008)	Rat mixed glia	SNRI (Venlafaxine)	IL-6	Decrease
			IFN-γ	Decrease
			TGF-β	Increase
			IL-10	No change

(Table 2) contd....

Study	Cell Used	Antidepressants	Target Studied	Result
Ha *et al.* (2006)	Mouse microglia (BV2)	SSRI (Fluoxetine)	NO	Increase
			iNOS mRNA	Increase
			IL-6 mRNA	Increase
			TNF-α mRNA	Increase
			NF-κB activity	Increase

that treatment with those antidepressants for 24 h significantly inhibited the secretion of both cytokines, but did not change the expression of the mRNAs [41].

We previously studied the effects of various types of antidepressants, as well as the mood stabilizer lithium chloride, on the release of the pro-inflammatory mediators IL-6 and NO from IFN-γ-activated murine 6-3 microglial cells by using ELISA and the Griess reaction, respectively [42]. We showed that 24-h pretreatment with the TCA imipramine, the SSRI fluvoxamine or the SNRI reboxetine significantly inhibited IL-6 and NO production in a dose-dependent manner. On the other hand, lithium chloride had a different spectrum of action, namely by enhancing IFN-γ-induced IL-6 production and inhibiting NO production.

Vollmar *et al.* (2008) measured IL-6, IL-10, IFN-γ and TGF-β concentrations in an astroglia-microglia co-culture treated with venlafaxine for 16 h by ELISA assay [43]. The culture system they employed allows mimicking of an inflammatory milieu by increasing the cultured microglial fraction without any inflammatory stimuli. They demonstrated an augmentation of TGF-β release with a concomitant reduction in the secretion of IL-6 and IFN-γ. Furthermore, they found a significant change of microglial phenotype from activated to resting morphology.

In contrast to those studies, Ha *et al.* (2006) demonstrated that treatment of murine microglial BV_2 cells with fluoxetine resulted in significant increases in NO and in the mRNAs of inducible NO synthase (iNOS), IL-6 and TNF-α [44]. They furthermore showed that fluoxetine increased the DNA binding activity of transcription factor nuclear factor-κB (NF-κB), whose activation mediates inflammatory responses. However, the study did not measure the concentrations of IL-6 and TNF-α released from microglial cells. Based on this study and the study by Obuchowicz *et al.*, it can be presumed that antidepressants inhibit the glial secretion of pro-inflammatory cytokines but do not decrease their mRNA levels. Thus, antidepressants may induce post-transcriptional changes in pro-inflammatory cytokines or increase their degradation as Obuchowicz *et al.* suggested.

Although the majority of studies have shown that antidepressants of various classes decrease the glial production of pro-inflammatory cytokines and increase the anti-inflammatory cytokine production, the limitation of such *in vitro* studies should be addressed. Considering the fact that antidepressant treatment needs at least 10-14 days for any clinical effectiveness to appear, the treatment of glial cells with antidepressants for 16-24 h appear to reflect only acute effects of the drugs. In addition, we should note the antidepressant concentrations those studies employed. Maes *et al.* (1999) indicated that 1 μM corresponds to the plasma concentrations attained during clinical treatment [45]. Pharmacokinetic studies in animals have shown that the concentrations of antidepressants detected in certain organs such as the brain and spleen are 10-20 times higher than plasma concentrations due to the lipophilic property of antidepressants [46, 47]. Nevertheless, the concentrations 50-100 μM used in some *in vitro* studies seem to be rather higher than clinically relevant concentrations.

POSSIBLE MECHANISMS OF ANTI-INFLAMMATORY ACTIONS OF ANTIDEPRESSANTS

The exact mechanism by which antidepressants exert anti-inflammatory effects remains to be elucidated. Although one should remember the possible differences between the mechanism underlying anti-inflammatory effects of drugs *in vitro* and *in vivo*, several mechanisms are possible.

One of the most plausible involves an increase in intracellular cyclic adenosine monophosphate (cAMP) levels. A number of *in vivo* studies have shown that many antidepressants increase intracellular concentrations of cAMP through activation of monoamine receptors such as the receptors for 5-HT and NA [48, 49]. Also, *in vitro*, data indicate that antidepressants of several classes increase intracellular cAMP levels [50, 51]. We demonstrated that TCA, SSRI and SNRI inhibited IFN-γ-induced microglial production of IL-6 and NO *in vitro*. These inhibitions were reversed by the cAMP inhibitor SQ 22536 and by the protein kinase A (PKA) inhibitor Rp-adenosine3', 5'-cyclic monophosphorothioate triethylammonium salt (Rp-3', 5'-cAMPS), suggesting that the anti-inflammatory effects of various antidepressants on microglia are at least partially mediated by the cAMP-dependent PKA pathway [42]. These results are consistent with findings in a study on human whole blood [52]. We also demonstrated that lithium chloride reduced IFN-γ-induced microglial production of NO. Interestingly, the inhibition by lithium chloride was not reversed by either SQ 22536 or Rp-3', 5'-cAMPS, indicating such an inhibitory effect of lithium chloride is not mediated by the cAMP/PKA pathway.

In a number of cell types, the activation of the cAMP/PKA pathway has been shown to inhibit NF-κB activity [53], whose activation is known to induce the gene expression of iNOS and various pro-inflammatory cytokines. Specifically, in rat primary astrocytes [54] and human monocytes [55], the activation of the cAMP/PKA pathway

inhibits LPS-mediated induction of NF-κB binding activity. Activation of the cAMP/PKA pathway not only down-regulates NF-κB activity, it also down-regulates the Janus family kinase (JAK)/signal transducer and activator of transcription (STAT) 1 pathway. Upregulation of the pathway is known to transactivate IFN-γ-responsive genes including iNOS [56] and IL-12 [57]. Recently, Delgado *et al.* (2003) demonstrated in mouse microglia that vasoactive intestinal peptide inhibited IFN-γ-induced JAK/STAT1 activation through upregulation of the cAMP/PKA pathway [58]. Therefore, antidepressant induced upregulation of the cAMP/PKA pathway may mediate inhibitory effects of antidepressants on LPS or IFN-γ-evoked inflammatory transactivations in immune cells (Fig. **1**).

The manner in which *in vivo* anti-depressant treatment increases intracellular cAMP levels appears to be strait forward. Explicitly, it is believed that antidepressants increase synaptic levels of 5-HT and NA through inhibiting reuptake by their transporters on presynaptic neurons. Thus causes activation of their receptors which are coupled to G proteins that can regulate the cAMP system. Through G-protein activation of adenylate cyclase (i.e., through the activation of 5-HT or NA receptor subtypes positively coupled to adenylate cyclase), cAMP production is increased.

It remains unclear as to how antidepressants increase intracellular cAMP levels *in vitro*. Antidepressants may act on cells *in vitro* independently of monoamine receptors coupled to G proteins. A recent genetic study has shown that genes of phosphodiesterases (PDEs), which degrade cAMP, are associated with a susceptibility to major depression and to antidepressant treatment response [59]. Accordingly, antidepressants may directly affect PDE functions in cells and thus increase the intracellular cAMP *in vitro*. Alternatively, we can presume that antidepressants could have direct effects on G proteins.

Maes and colleagues hypothesized that the mechanism is related to the effect of antidepressants on the serotonergic system by 5HT influencing cytokine production [30, 60]. Obuchowicz *et al.* suggested that the mechanism might be nonspecific because antidepressants are potent inhibitors of sodium and calcium influx [61]. Further studies on this subject are clearly warranted.

POTENTIAL OF ANTIDEPRESSANTS AS PREVENTIVES AGAINST ALZHEIMER'S DISEASE

Dementia and major depression are frequently comorbid among elder people. There is enough evidence from epidemiologic and neuropsychologic studies that major depression is associated with AD, even though it is uncertain whether major depression represents an early sign of dementia or a risk factor for dementia (for review see [62]).

It is well established that inflammatory processes are closely associated with the pathogenesis of a broad spectrum of neurodegenerative diseases [63, 64]. In AD, senile plaque is one of the neuropathological hallmarks of AD and a site of inflammatory processes, as evidenced by the presence of degenerating neurons and numerous reactive microglia and astrocytes [65, 66]. A number of *in vitro* studies have shown that amyloid-β-activated microglia damage or kill neurons by the release of inflammatory mediators such as pro-inflammatory cytokines, nitric oxide and superoxide radicals [67-70]. Therefore, chronic inflammation may be involved in the pathogenesis of both major depression and dementia.

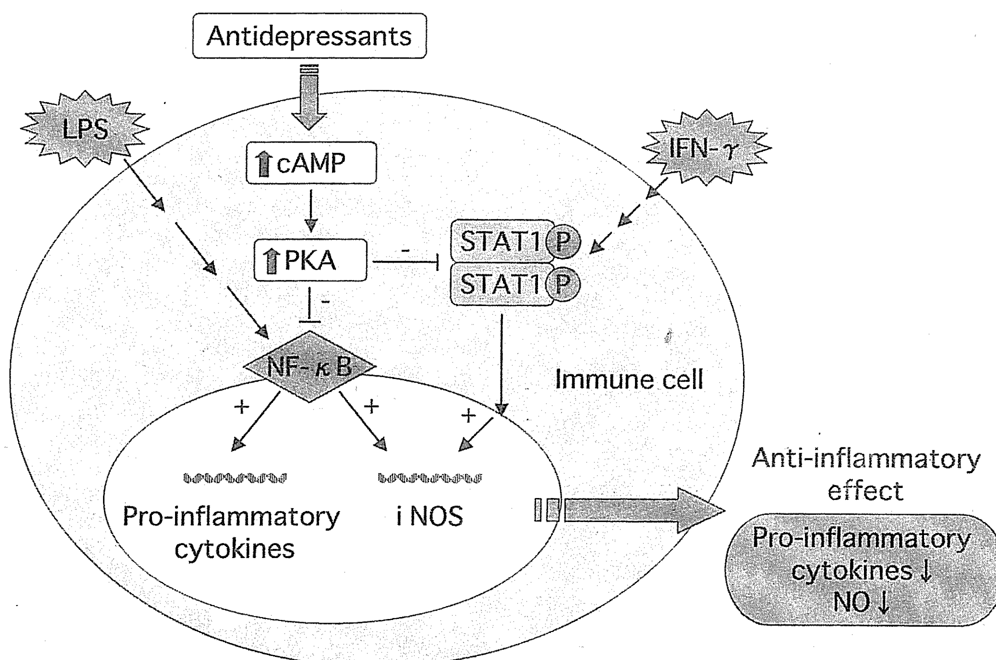

Fig. (1). Scheme for possible mechanism by which antidepressants exert anti-inflammatory effect in immune cells. Antidepressants may inhibit LPS or IFN-γ-evoked inflammatory transactivations through the up-regulation of cAMP/PKA pathway in immune cells. See text for details.

More than twenty epidemiological studies have shown that individuals are relatively spared from AD if they have been taking nonsteroidal anti-inflammatory drugs (NSAIDs) or have suffered from conditions where such drugs are routinely used (for review see [71]). In this regard, it is tempting to speculate that antidepressants with anti-inflammatory effects could be useful treatments for neurodegenerative diseases including AD. Interestingly, pre-symptomatic and chronic treatment with paroxetine has been shown to decrease AD-like pathology and reverse memory impairments in 3x transgenic AD mice [72]. Furthermore, in a small, 8-week double-blind placebo-controlled clinical study, fluoxetine was effective in reducing cognitive decline and behavioral abnormalities in patients with mild cognitive impairment [73]. This suggests that antidepressants could ameliorate AD or inhibit the progression of major depression to dementia.

It should be noted that such positive effects of antidepressants on memory and cognitive impairment might not be due to anti-inflammatory effects. Experimental evidence shows that chronic treatment with various antidepressants enhances neurogenesis in adult hippocampus [74, 75]. Clinical evidence indicates that long-term paroxetine treatment increases memory and hippocampal volume in patients with post traumatic stress disorder [76]. Accordingly, improvement of memory and cognition in the aforementioned two studies might be due to the hippocampal neurogenesis induced by antidepressants. Nevertheless, the anti-inflammatory properties of antidepressants may still be involved since the inflammation associated with LPS-activated microglia has been demonstrated to suppress hippocampal neurogenesis in adult rats [77].

CONCLUSIONS

Accumulating evidence indicates that major depression is associated with inflammation and that various types of antidepressants possess anti-inflammatory properties even though the exact mechanisms remain to be elucidated. Association between major depression and neurodegenerative diseases including AD may be based on the importance of chronic inflammation in the pathogenesis. Some preliminary studies support the hypothesis that antidepressants could prevent AD or inhibit the progression of major depression to dementia. Further studies along these lines are clearly warranted, even though careful consideration of the side effects of antidepressants is required in studies on aged people.

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