ARTICLE

Differential effect of lithium on spermidine/spermine N1-acetyltransferase expression in suicidal behaviour

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Abstract

An altered polyamine system has been suggested to play a key role in mood disorders and suicide, a hypothesis corroborated by the evidence that lithium inhibits the polyamine mediated stress response in the rat brain. Recent post-mortem studies have shown that spermidine/spermine N1-acetyltransferase (SAT1), the key regulator of cellular polyamine content, is under-expressed in brains from suicide victims compared to controls. In our study we tested the effect of *in vitro* lithium treatment on SAT1 gene and protein expression in B lymphoblastoid cell lines (BLCLs) from bipolar disorder (BD) patients who committed suicide (and for which BLCLs were collected prior to their death), BD patients with high and low risk of suicide and a sample of non-psychiatric controls. Baseline mRNA levels were similar in the four groups of subjects ($p > 0.05$). Lithium had no effect in suicide completers ($p > 0.05$) while it significantly increased SAT1 expression in the high risk ($p < 0.001$) and low risk ($p < 0.01$) groups as well as in controls ($p < 0.001$). Protein and mRNA levels were not correlated; lithium significantly reduced protein levels only in the control sample ($p < 0.05$). Our findings suggest that SAT1 transcription is influenced by lithium and that this effect is altered in BD patients who completed suicide, further supporting a role for polyamines in suicide.

Introduction

Suicide is among the 10 leading causes of death in the general population (Bertolote and Fleischmann, 2005).

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It has been estimated that 90% of individuals who commit or attempt suicide are affected by psychiatric disorders, with 50–60% of these subjects suffering from major affective disorders (MAD; Beautrais et al., 1996; Rihmer and Kiss, 2002; Rihmer, 2007). Although suicide is not a direct consequence of MAD, the presence of family history of mood disorders and suicidal behaviour is considered among the most significant predictors of suicide risk (Brent et al., 2002, 2004; Mann et al., 2005). A work by

Cavazzoni et al. (2007) showed that in families of MAD probands the risk of suicide correlated with the number of affected family members and the number of subjects with history of suicidal behaviour. Families could be divided into three groups with low, intermediate and high suicidal risk, with the latter being associated with the higher number of first-degree relatives with MAD and history for suicidal behaviour.

The heritability of suicide has been demonstrated by twin and adoption studies showing higher concordance for suicide among monozygotic twins than dizygotic twins and higher suicide risk in biological relatives of suicide probands compared to adopted relatives (Roy et al., 1991; Roy and Segal, 2001; Voracek and Loibl, 2007). While this evidence suggests an important role for genetic predisposition, the genetic underpinnings of suicide are far from being completely understood.

To date, the majority of molecular studies have been focused on the serotonin system (Mann et al., 2001). However, efforts have so far failed to provide robust evidence toward specific genes, likely because of the complex interaction between genetic and environmental factors that, together with the complexity of the phenotype, may contribute to the inconsistency of findings.

Recent data have pointed to an altered activity of the polyamine system in suicide (Fiori and Turecki, 2008). Polyamines (agmatine, putrescine, spermine and spermidine) are ubiquitous molecules involved in cell growth and differentiation. They modulate neurotransmission and are responsible for the polyamine mediated stress response (PSR; Wallace et al., 2003; Igarashi and Kashiwagi, 2010). The PSR is a cascade of molecular events transiently activated by acute stress stimuli. However, chronic stress can lead to a hyperactivation of the polyamine system, ultimately leading to cell growth inhibition and cell death (Cousin et al., 1982; Paschen et al., 1988; Gilad and Gilad, 1992). Spermidine/spermine N1-acetyltransferare 1 (SAT1) is the key regulator of cellular polyamine content and is involved in the catabolism of spermidine and spermine, a key step in the maintenance of polyamine homeostasis (Pegg, 2008). Recently, Sequeira et al. (2006) showed a significant lower expression of SAT1 in multiple brain regions of suicide completers with and without depression compared to controls. The same finding was reported by further post-mortem studies in suicide (Sequeira et al., 2007; Guipponi et al., 2009; Klempan et al., 2009; Fiori et al., 2011) also showing altered expression for other polyamine system genes (Fiori et al., 2011). These data provide additional evidence for the involvement of altered polyamine homeostasis

in suicide. Moreover, it is of interest that in animal studies the PSR could be blocked by chronic lithium (Gilad and Gilad, 1996, 2003) a mood stabilizing drug with a well-established anti-suicidal effect (Bocchetta et al., 1998; Ahrens and Muller-Oerlinghausen, 2001).

Based on these findings, we investigated the effect of *in vitro* lithium treatment on *SAT1* gene and protein expression in B lymphoblastoid cell lines (BLCLs) derived from a group of bipolar disorder (BD) patients who died by suicide and for which BLCLs were collected prior to their death; two groups of BD patients with high and low risk of suicide and a group of non-psychiatric controls.

The aim of our study was to test whether under-expression of SAT1 was also present in peripheral tissue derived from subjects who died by suicide. Lithium treatment *in vitro* allowed us to explore whether this ion affected SAT1 expression and whether the effect was different in BLCLs from the four groups of subjects. The inclusion of two groups of BD patients with high and low risk of suicide allowed us to investigate whether SAT1 expression pattern of these groups differed from suicide completers.

Method

Sample

BD patients and healthy controls were selected from two existing clinical samples for which a bio-bank including DNAs and BLCLs had been set. Specifically, 30 patients were selected from the sample recruited at the Lithium Clinic of the Clinical Psychopharmacology Centre, Unit of Clinical Pharmacology of the University Hospital of Cagliari and of the Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology of the University of Cagliari; 15 patients were selected from a sample recruited through an ongoing genetic study and the Maritime Bipolar Registry, a community-based project in the Maritime Provinces of Canada (Hajek et al., 2005). In both samples, a lifetime consensus diagnosis according to Research Diagnostic Criteria (RDC; Spitzer et al., 1978) and DSM-IV criteria was obtained using personal semi-structured interviews [Schedule for Affective Disorder and Schizophrenia Lifetime Version (SADS-L); Endicott and Spitzer, 1978] and a systematic review of patients' medical records. First- and second-degree relatives were interviewed in person using the SADS-L and diagnoses were made according to RDC and DSM-IV criteria. In relatives unavailable for direct interviews, diagnosis was made using the Family History-Research Diagnostic Criteria

Table 1. Demographic and clinical variables of the sample

Variable		SC (n=9)	HR (n=19)	LR (n=17)	Con (n=21)	p value
Diagnosis	BD I	8 (89%)	14 (73%)	13 (76%)	–	0.656*
	BD II	1 (11%)	5 (27%)	4 (24%)	–	
Sex	M	4 (44%)	3 (16%)	5 (29%)	4 (19%)	0.348*
	F	5 (56%)	16 (84%)	12 (71%)	17 (81%)	
Age at sampling, yr±S.D.		41.6±14.9	42.0±10.0	41.0±10.3	37.3±9.2	0.767†
Lithium at sampling, n (%)		3 (33.3)	9 (47.4)	4 (23.5)	–	0.217*

SC, Suicide completers; HR, high risk; LR, low risk; Con, healthy controls; BDI, Bipolar disorder I; BDII, bipolar disorder II; M, male; F, female.

* χ^2 test.

† One-way analysis of variance.

(Andreasen et al., 1977). Information was gathered from at least two informants and from available medical records.

Specifically, we included nine subjects known to have died by suicide subsequent to being sampled for cell culture (Cagliari, $n=6$; Halifax, $n=1$) or who committed a potentially lethal suicide attempt and survived with resuscitation procedures that left subsequent brain damage (Halifax=2). Considering the severity of the suicide attempt and that no other subjects with similar lethality of the suicide act were present in the high risk sample, these subjects were grouped with suicide completers for this study. Two additional groups of patients defined as at low and high suicide risk were characterized according to the following criteria based on the findings of the Cavazzoni et al. (2007) study:

- Low risk (Cagliari, $n=12$; Halifax, $n=5$): subjects affected by BD with no prior history of suicidal behaviour and at least three first- or second-degree relatives with MAD, none of whom committed or attempted suicide.
- High risk (Cagliari, $n=12$, Halifax, $n=7$): subjects affected by BD with personal history of non-lethal suicide attempt and presence of at least one first- or second-degree relative with MAD who committed or attempted suicide.

Non-psychiatric controls were recruited among subjects with no personal and family history of psychiatric disorders and suicidal behaviour, based on direct assessment. For this study, we included 12 controls from the Cagliari sample and nine from the Halifax sample. None of the controls was taking any psychotropic medication at the time of blood sampling, while a proportion of BD patients were using lithium

(see Table 1 for more details). All subjects gave their informed consent to participate in the study and the local ethics committees approved the protocols.

Establishment of BLCLs

Blood was sampled for each patient at the time the informed consent was signed for the enrolment in BD genetic studies. BLCLs were established from fresh blood following standard procedures (Neitzel, 1986). Briefly, lymphocytes were separated and transformed using Epstein Barr virus. Cells were then cultured in RPMI 1640 medium containing 15% of foetal bovine serum, 1% of L-glutamine 200 mM, 1% of sodium pyruvate 100 mM and 1% penicillin/streptomycin in a humidified incubator at 37 °C in the presence of 5% CO_2. When log phase growth was reached, cells were pelleted and suspended in foetal bovine serum with 10% dimethylsulfoxide and stored in liquid nitrogen.

BLCLs regrowth and treatment

For the present study, BLCLs were thawed from liquid nitrogen and grown under controlled conditions to limit the effect of variability on gene and protein expression levels. Each cell line was passaged minimally to obtain the required cell count ($5–8 \times 10^6$). The viability and number of cells was assessed using a Bürker cell counting chamber and Trypan Blue. After reaching the required cell count, each BLCL was divided into two lines and cultured in medium with or without LiCl 1 mM for 7 d. Media was replaced every other day; at day 7 cells were pelleted by centrifugation and processed for total RNA and proteins extraction.

RNA and protein extraction

Total RNA and proteins were obtained from 6–9×10^6 cells. RNA was extracted using either Trizol (Life Technologies, USA) or RNeasy mini kit (Qiagen, Switzerland), following manufacturer's protocols. RNA was suspended in $500\,\mu l$ storage solution (Life Technologies) and quantified using a spectrophotometer. All RNAs had a 260:280 nm ratio between 1.8 and 2 and a 28 s:18 s rRNA ratio >2. An amount of $2\,\mu g$ RNA was treated with DNAse before real time polymerase chain reactions (PCRs).

Proteins were extracted from six subjects selected from each of the four groups of subjects of the Cagliari sample using the RNeasy mini kit (Qiagen) following manufacturer's procedures. Samples concentrations were determined using the 2D Quant-Kit (GE Healthcare, UK) and then stored at $-80\,^{\circ}C$ prior to electrophoresis.

Real-time PCRs

Relative expression of SAT1 was measured by using either a one- or two-step real time PCR. For samples processed with the two-step approach, cDNA was synthesized from $1\,\mu g$ total RNA using MMLV-RT and random decamers (Retroscript kit, Life Technologies). PCRs were carried out on an ABI Prism 7000 sequence detection system (Life Technologies). Reactions were performed in a $20\,\mu l$ final volume using 50 ng total RNA under the following conditions: 2 min at $50\,^{\circ}C$, 10 min at $94\,^{\circ}C$ and 45 cycles at $94\,^{\circ}C$ for 15 s and $60\,^{\circ}C$ for 1 min.

One-step real time PCRs were performed using the TaqMan EZ-RT-PCR Kit (Life Technologies). PCRs were run on a DNA Engine Opticon 2 (MJ Research, USA) using 50 ng total RNA in a final volume of $25\,\mu l$ under the following conditions: 2 min at $50\,^{\circ}C$, 30 min at $60\,^{\circ}C$, 5 min at $95\,^{\circ}C$ and 40 cycles at $94\,^{\circ}C$ for 20 s and $62\,^{\circ}C$ for 1 min.

All the real time PCRs were carried out using the TaqMan assay 'on demand' Hs00161511_m1 (Life Technologies), spanning the junction between exons 3 and 4, thus reducing the chance of detecting genomics DNA. β Glucuronidase (GUSB) and cyclophilin A (PPIA) were used as reference gens for normalization. The PCRs were performed in triplicate and threshold cycles (Ct) measured with either Sequence Detector Software v 2.2 (ABI Prism 7000) or Opticon Monitor v 3.1 (DNA Engine Opticon 2). Ct values for each individual were calculated as the mean of the three replicated measures of the gene ratio to the reference gene.

The efficiency of the PCRs for SAT1, GUSB and PPIA was measured using the Ct slope method with a 10-fold dilution curve. The efficiency of all the assays was >90%, thus allowing the use of the comparative Ct method ($2^{-\Delta\Delta Ct}$) for relative quantification of SAT1 using cDNA from an external control as a calibrator.

Protein quantification

Samples were analysed by mono and two-dimensional Western blotting. One and 2D-blot were incubated with anti-sat1 antibody (Abnova, Taiwan) and developed with ECL Advance (GE Healthcare, Sweden). Membranes were acquired using a LAS-1000 apparatus (Fujifilm, Japan). Blot images were imported into TotalLab TL120 (for 1D gel) and Progenesis SameSpots 3.3 (Nonlinear Dynamics, UK). All band and spots were manually reviewed and validated to ensure proper detection and matching. After matching across gels, spot volumes were exported as log standardized abundance.

Statistics

Differences in demographic and clinical variables between groups were tested with χ^2 test or analysis of variance (ANOVA), respectively. Normality of the distribution of expression values was verified with the Shapiro–Wilk test. Repeated measures ANOVA was performed to estimate the effect of treatment in the individual subject groups stratified by the Centre (Cagliari, Halifax) and their interactions. The effects of treatment in each group were tested *post hoc* using the paired *t* test corrected for multiple comparisons (Bonferroni's method).

Differences between groups in the mean fold changes (FC) for lithium effect was evaluated with one-way ANOVA and Tukey's multiple comparison test.

Significance threshold was set at $p < 0.05$, two-sided. The analyses were performed using SPSS statistical software package v20 (SPSS, Inc., USA) and GraphPad Prism v5 (GraphPad Software Inc., USA).

Results

The demographic and clinical characteristics of the sample are summarized in Table 1. The four groups of subjects and the two centres (Cagliari and Halifax) did not differ for any of the variables tested ($p > 0.05$).

Relative expression levels of SAT1 were normally distributed in all the groups (Shapiro–Wilk test, $p > 0.05$). Repeated measures ANOVA showed a trend for a differential expression of SAT1 between groups ($F=2.376$; $p=0.08$, Table 2), while the effect of lithium treatment was highly significant ($F=38.65$, $p < 0.0001$)

Table 2. Results from repeated measures ANOVA in the combined and individual samples

Combined sample			Cagliari		Halifax	
	F	p value	F	p value	F	p value
Within subjects						
Treatment	38.65	0.000	42.59	0.000	8.69	0.008
Treatment × Group	5.91	0.001	2.740	0.057	2.71	0.072
Treatment × Centre	0.16	0.687	–	–	–	–
Between subjects						
Group	2.37	0.081	0.510	0.672	2.69	0.074
Centre	2.15	0.168	–	–	–	–

F statistics from the analysis of variance (ANOVA) test with associated p values.

across both centres. Importantly, lithium effect significantly differed between the four groups of subjects ($F=5.91$; $p=0.001$). In detail, lithium did not influence SAT1 expression only in the group of suicide completers ($p>0.05$) while it over-expressed the gene in the high risk (corrected $p<0.001$) and low risk (corrected $p<0.01$) groups as well as in healthy controls (corrected $p<0.001$; Fig. 1a, Table 2). The effect size of lithium treatment remained the same when the two samples were analysed separately (Fig. 1b, c, Table 2). The comparison of the mean FC of *in vitro* lithium effect showed a significant difference when comparing completers with the other groups (Fig. 2).

In contrast with the gene findings, protein levels were reduced by lithium treatment ($F=9.375$; $p=0.006$; Table 3). After Bonferroni's correction this effect remained significant only in healthy controls (corrected $p<0.05$, Fig. 3). The variable 'group' was also significant ($F=7.528$, $p=0.0015$), while the 'treatment' × 'group' interaction showed a trend for significance ($F=2.700$; $p=0.071$).

Discussion

In this study we explored the effect of *in vitro* lithium treatment on SAT1 in BLCLs derived from BD patients who committed suicide and collected prior to suicide completion, BD patients with high and low suicide risk and a group of non-psychiatric controls.

The aims of our study were to: (a) explore whether the down-regulation of SAT1 shown in brains of suicide completers by previous studies was observable in lymphoblasts; (b) test whether SAT1 expression was influenced by lithium treatment *in vitro* and how specific this effect was in relation to the risk

of suicide; (c) explore whether SAT1 expression levels correlated with suicide completion and risk of suicide.

Our major finding is that SAT1 expression is differentially regulated in BD patients who died by suicide. Lithium robustly increased SAT1 mRNA levels in the low and high risk groups as well as in healthy controls, but it had no effect in suicide completers. This result indicates that SAT1 transcription regulation may be specifically altered in BD patients who commit suicide. Our findings are of particular interest in light of the previously replicated evidence of SAT1 down-regulation in brains from suicide completers. While more data are needed to clarify this effect, we speculate that, if an altered polyamine system is present in suicide, patients who commit suicide might not benefit from the polyamine-mediated lithium effect. The understanding of the mechanism through which lithium differentially regulates SAT1 would be of great help in understanding the contribution of the polyamine system in suicide. An effect of lithium on polyamines was already described by previous animal studies showing that chronic treatment before dexamethasone test blocks the PSR in the brain (Gilad et al., 1992; Gilad and Gilad, 1996). This effect appears to be at least in part determined by the inhibition of ornithine decarboxylase (ODC; Gilad and Gilad, 1992), the enzyme responsible for the synthesis of putrescine. This inhibition is not a direct effect, as demonstrated by the evidence that treating tissue homogenates with lithium does not block ODC activity (Gilad and Gilad, 1992). Lithium shows the same effect on two other polyamine enzymes, *S*-adenosylmethionine decarboxylase and SAT1. It is reasonable to hypothesize that, as for ODC, lithium does not directly interact with SAT1.
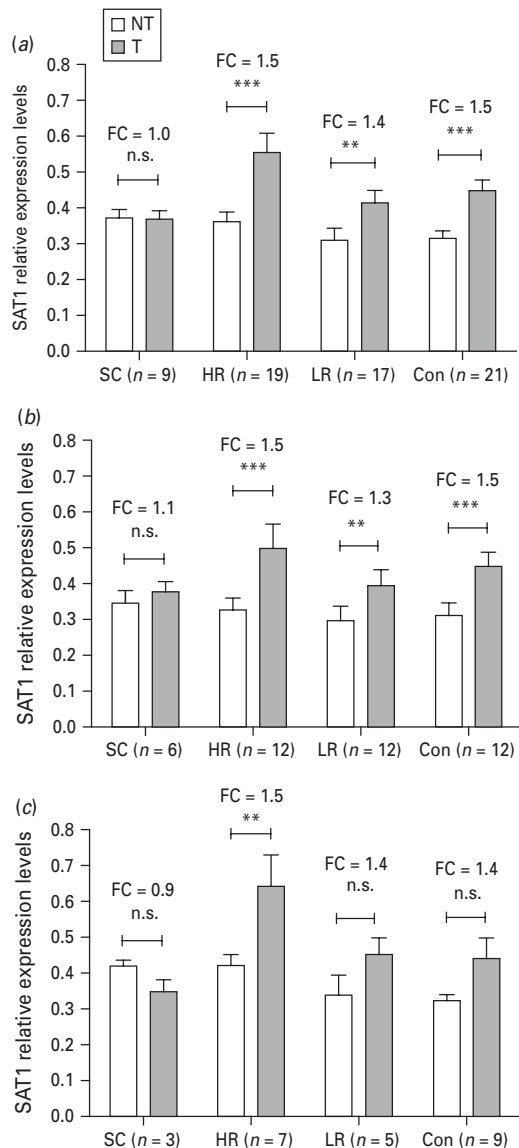
Fig. 1. Relative expression levels of spermidine/spermine N1-acetyltranserare 1 (SAT1; mean±s.e.) in B lymphoblastoid cell lines (BLCLs) treated (T) or not treated (NT) with LiCl from the three groups of bipolar patients with different risk of suicide and healthy controls (Con) in the combined sample (*a*) and in the two separate samples (*b*: Cagliari and *c*: Halifax). Treatment with LiCl had the same effect size in the combined and in the two samples. *p* values of *t* tests for treatment comparisons within groups were corrected with Bonferroni. n.s., *p*>0.05; ** *p*<0.01; *** *p*<0.001. SC, Suicide completers; HR, high risk; LR, low risk; FC, fold change.
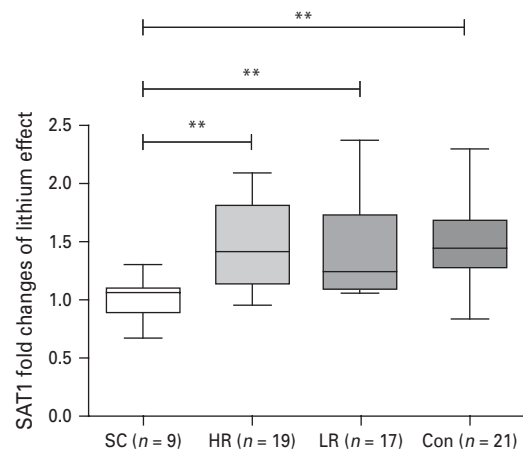
Fig. 2. Box plots with whiskers of the comparison between mean fold changes of the effect of *in vitro* lithium treatment of the four groups of subjects. Fold changes were obtained with the $\Delta\Delta Ct$ method by using the untreated B lymphoblastoid cell lines as the calibrator for each treated cell line. The one way analysis of variance test showed $F=4.58$ with $p=0.0059$. ** $p<0.05$ (Tukey's post test). SC, Suicide completers; HR, high risk; LR, low risk; Con, healthy controls.

inducible by several molecules and stressors (Pegg, 2008). The first step in the activation of SAT1 is the increase in mRNA levels, which is generally discrete while the large augmentation in its activity is the result of a combination of increased mRNA levels, stability, translational efficiency and stabilization of the SAT1 protein (Pegg, 2008). Thus, lithium may interfere with any of the mentioned steps, ultimately resulting in altered mRNA levels.

The differences in lithium-induced increase in SAT1 mRNA levels might be determined by sequence variants located in regulatory regions of the gene. Suicide completers may carry such variants predisposing them to a less inducible transcription. Previous findings have shown that the single nucleotide polymorphism (SNP) rs6526342, near the polyamine response element in the promoter region, predicts SAT1 expression and is associated with suicide completion (Sequeira et al., 2006; Fiori et al., 2009). An insertion/deletion located in the same region was shown to predict SAT1 expression and was associated with suicide in depressed patients (Fiori et al., 2009; Fiori and Turecki, 2010). In another study, Guipponi et al. (2009) failed to replicate the association between SNPs in the promoter region of SAT1, including rs6526342, and suicide. Further investigation of SAT1 sequence would help to better understand the correlation between gene mutations, expression levels and suicide.

The differences that we observed in lithium effect on SAT1 mRNA levels might be the consequence of events either upstream or downstream of SAT1. SAT1 activity is very low in resting cells but it is highly

Table 3. Results from the repeated measures ANOVA for SAT1 protein levels measured in six subjects selected from each of the three groups of bipolar patients with different risk of suicide and healthy controls of the Cagliari sample

Cagliari sample		
	F	*p* value
Within subjects		
Treatment	9.375	0.006
Treatment × Group	2.700	0.081
Between subjects		
Group	7.528	0.002

F statistics from the analysis of variance (ANOVA) test with associated *p* values.

Fig. 3. Protein levels of spermidine/spermine N1-acetyltranserare 1 (SAT1; mean±S.E.) in B lymphoblastoid cell lines (BLCLs) treated (T) or not treated (NT) with LiCl from six subjects selected from the Cagliari sample. After Bonferroni's correction, the effect of LiCl was marginally significant only in the sample of controls. n.s., $p > 0.05$; * $p < 0.05$. SC, Suicide completers; HR, high risk; LR, low risk; Con, healthy controls; FC, fold change.

In contrast with previous post-mortem studies, we reported no differences in SAT1 mRNA levels between completers and controls. However, while polyamines are ubiquitous molecules, their activity and levels are known to significantly differ between tissues. In animal studies, chronic intermittent application of stressors causes a recurrence of PSR in the brain but it leads to habituation of the response in the periphery (Gilad and Gilad, 2003). Moreover, acute administration of lithium blocks the dexamethasone induced increase in ODC activity in the liver but not in the brain (Gilad and Gilad, 1992; Gilad et al., 1994). The brain polyamine system is involved in the response of the central nervous system (CNS) to stress stimuli and it is likely that patients with psychiatric disorders or suicidal behaviour have more prominent alterations at the level of the CNS than in other tissues. On the other hand, we can hypothesize that basal SAT1 transcriptional activity could be sufficient to guarantee optimal polyamine functioning in peripheral tissues and only specific stimulations, like lithium in our case, could elicit the underlying alterations observed in suicide completers.

Another finding of our study is that the effect of lithium did not correlate with the familial risk of suicide, as suggested by the similar expression levels and effect size in the low risk and high risk groups. While BD subjects with suicidal behaviour could represent a distinct subgroup of BD patients (Slama et al., 2004), polyamine functioning may not differ significantly between attempters and non-attempters. On the other hand, in our study, lithium effect on SAT1 was different in completers compared to the high risk group, suggesting that completers may constitute a more extreme phenotype with a more prominent alteration in SAT1 transcription activity. The question
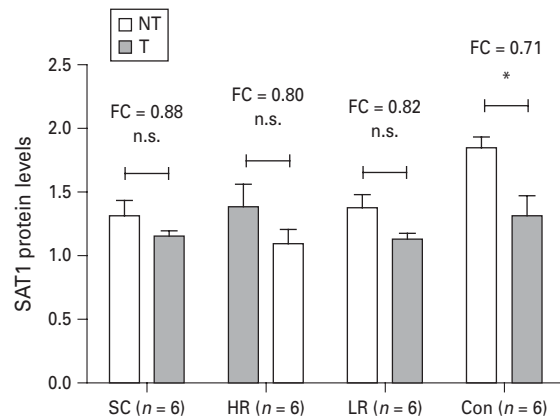
whether suicide completers and attempters represent two different subgroups of the same phenotype has been addressed by many authors, but the question is still open. Studies comparing suicide completers and attempters have shown that these two groups of subjects have not only many clinical and demographic similarities but also important differences (Linehan, 1986; Beautrais, 2001, 2002; Fushimi et al., 2006; DeJong et al., 2010). Genetic studies would be of great help in dissecting diversities and commonalities in the molecular underpinnings of these two groups.

In our study, lithium had no differential effect in BD patients (with the exception of completers) compared to healthy controls. This result suggests that altered functioning of SAT1 may be at least partially independent from BD illness. This is in line with previous post-mortem studies reporting significant under-expression of SAT1 in brains from suicide completers with and without mood disorders compared to healthy controls, although the difference was more significant in suicide completers with than without mood disorders (Sequeira et al., 2007; Klempan et al., 2009).

In regard to the protein study, we reported no differences in lithium effect among groups. Lithium decreased SAT1 levels, with a more prominent effect in healthy controls, while it had a similar effect in the three groups of BD patients with different suicidal behaviour. A direct correlation between SAT1 mRNA and protein levels was previously reported by Sequeira et al. (2006) in brains from suicide completers

compared to controls. However, as mentioned earlier, the different tissues studied may have accounted for the discrepancy with our findings.

The difference in lithium effect between gene and protein levels that we observed could be determined by the complex sequence of post-transcriptional and post-transductional modifications of SAT1 that is also known to differ according to tissues (Pegg, 2008). Further investigation would be needed to dissect the correlation between gene and protein expression in different tissues.

To the best of our knowledge, our study is the first reporting alterations of SAT1 in a peripheral tissue from suicide completers. Significantly, differences in polyamine levels and enzyme activity have been shown in plasma and serum from psychiatric patients (Fiori and Turecki, 2008), but none of these studies investigated SAT1. The analysis of the polyaminergic metabolism as well as of polyamines gene and protein expression in peripheral tissues from subjects with suicidal behaviour could represent a powerful tool for the identification of biomarkers. While this approach may be performed with relative feasibility in living patients characterized for suicidal risk, it is more difficult to carry out on subjects who died by suicide. Indeed, the analysis of the correlation between clinical variables and molecular determinants would require detailed information, such as records on the clinical course, psychotropic medications and past history of childhood/sexual abuse that are generally unavailable or difficult to gather for suicide victims. In this regard, our study has the advantage of using lymphoblasts collected prior to death from patients systematically followed in specialized clinics and for whom detailed clinical information is available. Moreover, lymphocytes are easy to collect from living patients and can be used as a model for testing the effect of drugs *in vitro*, thus representing a tool for pharmacogenetic studies and diagnostic tests. Lymphoblasts can also potentially overcome some of the limitations characterizing post-mortem studies, such as the difficulty in tissue sampling, diagnosis and substance abuse or pharmacological treatments at the time of death that could influence gene and protein expression (Dowlatshahi et al., 1999; Lipska et al., 2006; Halim et al., 2008).

In conclusion, our findings suggest that lithium affects differentially SAT1 mRNA levels in lymphoblasts from BD patients who completed suicide, thus suggesting an altered transcription regulation of this gene in suicide. Further analysis aimed at the identification of elements and processes involved in such differential transcription activity would help in better understanding the role of the polyamine system in suicide. Moreover, the analysis of the correlation between peripheral and brain SAT1 expression and activity in subjects who commit suicide would be of great help in the search for peripheral biomarkers of suicide.

Acknowledgements

Statement of Interest

None.

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