Lack of effect of mood stabilizers or neuroleptics on GSK-3β protein levels and GSK-3 activity

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Abstract

Glycogen synthase kinase (GSK)-3β protein levels and GSK-3 activity were previously found to be over 40% reduced in the post-mortem prefrontal cortex of schizophrenic patients. Lithium and valproate have been reported to selectively inhibit GSK-3. We hypothesized that in-vivo administration of lithium and valproate would result in up-regulation of GSK-3β protein levels and GSK-3 activity. The present study aimed to evaluate the possible involvement of neuroleptic treatment in the decrease of GSK-3 in schizophrenia. Rat frontal cortex GSK-3β protein levels and GSK-3 activity were measured following administration of therapeutic doses of lithium or valproate for 11 d, or of haloperidol, chlorpromazine or clozapine for 21 d. None of the drugs induced a change in GSK-3β protein levels. All the drugs except chlorpromazine (which was not tested) did not affect GSK-3 activity. This suggests that GSK-3β inhibition by lithium or valproate does not induce regulation of protein levels or activity and that the reduction in GSK-3β protein levels and GSK-3 activity in the post-mortem prefrontal cortex of schizophrenic patients is not neuroleptic-treatment related.

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Introduction

Glycogen synthase kinase (GSK)-3β is an important kinase widely expressed in the brain (Lau et al., 1999). GSK-3β plays a crucial role in the signalling processes associated with dysfunction in psychiatric disorders such as bipolar disorder and schizophrenia (Chen et al., 1999; Klein and Melton, 1996; Kozlovsky et al., 2000, 2001; Stambolic et al., 1996). Lithium and valproate, therapeutic agents for bipolar disorder, selectively inhibit GSK-3β activity within the therapeutic range (Chen et al., 1999; Klein and Melton, 1996; Stambolic et al., 1996). The mechanism by which these chemically dissimilar agents exert their inhibition on GSK-3 is not fully clear. However, many central inhibitory processes induce negative feedback resulting in up-regulation of the expression of the drug target that compensates for a decrease stimulation of the system. We therefore hypothesized that in-vivo lithium or valproate treatment may up-regulate GSK-3β protein levels.

GSK-3β has also been implicated in schizophrenia. We have previously reported reduced GSK-3β protein levels (Kozlovsky et al., 2000) and GSK-3 activity (Kozlovsky et al., 2001) in post-mortem prefrontal cortex of schizophrenic patients. The cause of this reduction could be disease related or treatment related.

In order to evaluate whether GSK-3 levels are affected by mood stabilizers or by neuroleptics, rats were administered lithium or valproate for 11 d, or chlorpromazine, haloperidol and the atypical neuroleptic clozapine for 21 d and frontal cortex GSK-3β protein levels and total GSK-3 activity measured.

Materials and methods

Sprague-Dawley rats (Harlan, Jerusalem) weighing 200–250 g at the start of the experiment were housed in a rat colony room with constant temperature (22–23 °C) and a 12 h on-off light–dark cycle. Food and water were provided ad libitum and contained drugs as described below. Each experiment consisted of 10 control and 10 treated rats housed 5 per cage.
The Ben-Gurion University Medical School Review Committee for the Use of Animals approved the experimental protocol and the procedures are in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Lithium chloride was administered in ground food (2 g/kg) and valproate in drinking water (12 g/l) (supplemented with 300 mg/l sodium saccharin) for 11 d. Lithium blood levels prior to sacrifice were 2.1 ± 0.2 (s.e.m.) mmol/l. Ten mg/kg of haloperidol were injected i.p. daily for 21 d as a solution in 0.2% citric acid. Control rats were injected with citric acid only. Chlorpromazine and clozapine were also administered in drinking water (10 mg/kg each) for 21 d. Rats treated in food or in drinking water were sacrificed on the last day of drug administration. Haloperidol-treated rats were sacrificed 24 h after the last injection. Sacrificed rats were decapitated, brains removed, frontal cortex specimens dissected and frozen at −80°C until processed. The drug doses administered were based upon a previous intensive study in our laboratory in which it was shown that these regimes result in rat blood levels within the therapeutic range in patients (Shaldubina et al., 2002). Lithium chloride and valproate were from Sigma (St. Louis, MO, USA), haloperidol from Janssen-Cilag (Latina, Italy), chlorpromazine from Taro (Haifa) and clozapine from Novartis (Basle). GSK-3β protein levels were determined by Western-blot analysis using specific antibodies for GSK-3β (Transduction Laboratories, Lexington, KY, USA). Sodium-dodecyl sulphate–polyacrylamide gel electrophoretic separation (SDS-PAGE) and immunoblotting of GSK-3β were preformed using a previously described procedure (Kozlovsky et al., 2000). Briefly, to minimize the effect of interblot variability, a calibration standard curve of known amounts of recombinant GSK-3β units (Upstate Biotechnology, New York, USA) was run in each gel and each sample was analysed at least three times and at two different protein concentrations (0.3 and 0.6 μg total protein), both within the linear range of detection. The absolute GSK-3β value (in units) of each band was derived from the standard curve run on the same gel. The mean value for each sample was calculated from all the replicates. GSK-3 activity was determined as previously described (Kozlovsky et al., 2001). Briefly, the assay was performed in a final volume of 16 μl, containing 10 μg protein, 100 μM of the GSK-3 specific substrate phospho-CREB (New England Biolabs Inc., Beverly, MA, USA), 100 μM [γ-32P]ATP (500–3000 cpm/μmol), 15 mM MgCl₂. In addition to the use of phospho-CREB as a specific substrate of GSK-3, in order to distinguish GSK-3 activity from non-specific kinase activity and to avoid the use of immunoprecipitation in screening multiple crude cell lysates, the reaction was carried out in the presence and absence of 30 mM LiCl (Ryves et al., 1998, 2001). Lithium ions specifically inhibit GSK-3 (Klein and Melton, 1996; Stambolic et al., 1996). Thus, GSK-3 activity was calculated as the difference between the value in the absence and in the presence of lithium. The difference, which represents GSK-3 activity, comprised 30–40% of the total kinase activity. This method does not discriminate between GSK-3α and GSK-3β and therefore measures total GSK-3 (α + β) activity. The results presented are only of those specimens for which at least two separate measurements could be conducted. Each single assay was carried out in triplicate.

### Table 1. GSK-3β protein levels and total GSK-3 activity in rat frontal cortex following administration of mood stabilizers or neuroleptics (results are means ± S.E.M.)

<table>
<thead>
<tr>
<th>Drug</th>
<th>GSK-3β Protein levels (μU/μg protein)</th>
<th>GSK-3 activity (pmol/mg protein × min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None*</td>
<td>476 ± 73 (n = 40)</td>
<td>21.3 ± 6.4 (n = 25)</td>
</tr>
<tr>
<td>Lithium</td>
<td>542 ± 75 (n = 10)</td>
<td>25.0 ± 7.2 (n = 8)</td>
</tr>
<tr>
<td>Valproate</td>
<td>459 ± 47 (n = 10)</td>
<td>29.9 ± 8.3 (n = 7)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>566 ± 96 (n = 10)</td>
<td>25.5 ± 6.3 (n = 9)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>498 ± 55 (n = 10)</td>
<td>20.0 ± 5.1 (n = 8)</td>
</tr>
<tr>
<td>CPZb</td>
<td>465 ± 107 (n = 10)</td>
<td>ndc</td>
</tr>
</tbody>
</table>

* Pooled controls.

b CPZ, chlorpromazine.

c nd, Not determined.

### Figure 1. A representative immuno blot and the corresponding calibration curve of rat frontal cortex GSK-3β protein levels following administration of lithium (Li), valproate (VPA), chlorpromazine (CPZ), haloperidol (HAL) and clozapine (CLZ) at concentrations and for the duration indicated in the Materials and methods section.
Results

Figure 1 depicts a representative immunoblot experiment of the effect of lithium, valproate, chlorpromazine, haloperidol and clozapine on GSK-3β protein levels in rat frontal cortex. As summarized in Table 1 none of the drugs induced a change in GSK-3β protein levels. All the drugs except chlorpromazine (which was not tested) did not affect total (α+β) GSK-3 activity.

Discussion

In vitro, lithium and valproate inhibit GSK-3 activity (Chen et al., 2000; Klein and Melton, 1996; Stambolic et al., 1996). The $K_i$ for lithium is in the range of 1–2 mM (Klein and Melton, 1996; Stambolic et al., 1996), at or above therapeutic concentrations in bipolar patients. As recently proposed (Grimes and Jope, 2001), this would result in lessening GSK-3β-mediated signalling cascades but not in their complete blocking (Jope, 1999). In general, receptors are up-regulated following exposure to their antagonists (Hauesler, 1990; Ridley et al., 2001). Among enzymes, some (i.e. tyrosine hydroxylase) but not all (i.e. monoamine oxidase) show increased activity following their inhibition (Kumer and Vrana, 1996; Murphy et al., 1975). In the present study, in-vivo inhibition of GSK-3β activity by lithium or valproate (Manji, 2001) was hypothesized to result in a compensatory up-regulation of its expression, which would support the concept that the molecular mechanism of mood stabilization involves GSK-3β. The results showing lack of effect of subchronic in-vivo lithium and valproate treatment on frontal cortex GSK-3β protein levels and total GSK-3 activity in rats reject our hypothesis. A similar lack of up-regulation has been reported for lithium's effect on adenylate cyclase. Ebstein et al. (1980) treated rats with lithium or 4 d respectively before sacrifice. The expected increase in noradrenaline-induced cyclic-AMP levels and basal adenylate-cyclase activity was not found. GSK-3β protein levels in post-mortem frontal cortex of lithium- and/or valproate-treated bipolar patients compared to control subjects or to untreated subjects have been previously reported unaltered (Kozlovsky et al., 2000; Lesort et al., 1999). These findings further support the lack of effect of in-vivo lithium or valproate treatment on frontal cortex GSK-3β protein levels and activity.

Neuroleptic drugs are $D_2$ dopamine receptor antagonists (Duncan et al., 1999). Dopamine receptor signalling involves cyclic-AMP and protein kinase A (Piomelli and Di Marzo, 1993). Protein kinase A has recently been shown to directly phosphorylate GSK-3β (Li et al., 2000), although direct evidence for the involvement of GSK-3β in the dopaminergic system has not yet been shown. The lack of effect of the typical neuroleptics chlorpromazine and haloperidol or the atypical neuroleptic clozapine on rat frontal cortex GSK-3β protein level may suggest that the reduction in GSK-3β levels in the frontal cortex of schizophrenic patients is disease-related and not related to their treatment with neuroleptic drugs.

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References


