The glutamatergic system and its relation to the clinical effect of therapeutic-sleep deprivation in depression – An MR spectroscopy study

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Abstract

Rapid improvement of depressive symptoms occurs after the administration of the NMDA antagonist ketamine. Ketamine administration is accompanied by an increase in GLX (sum-peak of glutamate, glutamine (GLN) and GABA) and GLN in the brain, as measured by magnetic-resonance (MR) spectroscopy. In healthy subjects, we observed an increase in GLX and GLN levels after total sleep deprivation (TSD), which has a rapid antidepressant effects. We examined, if an increase in GLX or GLN is related to the therapeutic effect of TSD. We examined 13 patients with major depression by means of proton MR spectroscopy (field strength: 1.5 T) before and after 24 h of TSD. Two anatomical areas (dorsolateral prefrontal cortex (DLPC) and parieto-occipital cortex (POC)) were studied. In the DLPC TSD did not change GLX or its elements, whereas the total creatine and choline signal increased marginally. No change could be observed in the POC. For further exploration we took gender and the presence of vegetative characteristics of melancholic depression into account, i.e. the presence of early morning awakening, appetite and weight loss was taken into account, to define vegetative melancholia (VM). TSD led to an increase in GLX and GLN in the DLPC only of male patients. In patients with VM an increase in GLN occurred in this area. The low field strength limits the accuracy for GLX and GLN estimates. Despite the exploratory nature of the study, it nevertheless supports earlier data on the importance of glutamatergic neurotransmission and furthermore of gender and/or vegetative features in depression.

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1. Introduction

Recently a renewed interest in the glutamatergic system as a treatment option for major depression emerged by the finding that the N-methyl-D-aspartate (NMDA) antagonist ketamine leads to a rapid improvement of depressive symptoms (Zarate et al., 2006; Berman et al., 2000). Magnetic-resonance (MR) spectroscopy studies demonstrated in healthy subjects that ketamine leads to an increase in glutamine (GLN) concentration in the anterior cingulated cortex (Rowland et al., 2005). In a pilot study, we observed an increase in gamma-amino-butyric-acid (GABA) and GLN in the pontine region of healthy controls in response to total sleep deprivation (TSD) (Murck et al., 2002), which has not been studied in humans after ketamine administration. Ketamine administration and REM-sleep deprivation in cats lead to similar biological changes (Susic, 1976). This
leads to the hypothesis that both manipulations, which can lead to a rapid improvement in depressive symptoms, may share a similar biological mechanism.

It is known that major depression is associated with changes in GABAergic and glutamatergic neurotransmission (Krystal et al., 2002; Petty et al., 1995; Petty, 1995; Sanacora et al., 1999, 2004). A reduction of GABA and an increase in glutamate in comparison to controls has recently been reported for the occipital cortex, which was especially pronounced in patients with the melancholic subtype of depression (Sanacora et al., 2004). A reduction in GLX (containing glutamate, GLN and GABA) and glutamate in the anterior cingulate cortex (Auer et al., 2000) and the dorsolateral prefrontal cortex (Michael et al., 2003b) has been described in severely depressed subjects with melancholic features. A more recent study in patients with less severe symptoms, showed a reduction of GABA and GLX in prefrontal brain-regions in unmedicated patients with depression (Hasler et al., 2007). The reduction in GLX was normalized by electroconvulsive therapy (ECT) (Michael et al., 2003b). The same group described an increase in GLX after ECT also in the left amygdalar region (Michael et al., 2003a) and the cingulate region (Pfleiderer et al., 2003) in ECT responders. Therefore ECT, which is another rapidly acting antidepressant therapy, seems to act similar to ketamine administration and to TSD in its way to affect the glutamatergic system. The main objective of our study is therefore to address the question whether these changes in GLX and GLN are related to clinical improvement in patients with depression treated with TSD. The second objective is to determine, whether factors with a known influence on the outcome of depression are also of importance in this paradigm.

First, gender has been reported to influence the neurobiology (Murck et al., 2003; Antonijevic et al., 2000) and treatment outcome of depression (Kornstein et al., 2000; Nickel et al., 2003; Murck et al., 2003; Thase et al., 2005). Gender also determines a differential physiological response to sleep deprivation (Armitage et al., 2001; Corsi-Cabrera et al., 2003).

Second, melancholic characteristics seem also to affect changes of the GABA-glutamate system in depression (Sanacora et al., 2004). We refined the definition on the basis of the vegetative characteristics of melancholia (early morning awakening, appetite- and weight loss), which seems to differentiate distinct patient populations in terms of the neurobiology, in particular the activity of the hypothalamus–pituitary–adrenocortical system (Casper et al., 1987; Antonijevic et al., 2000; Künel et al., 2003) and treatment outcome (Murck et al., 2005a,b). In this context, it is important to note that the parameters relating to the clinical features melancholia are not independent from gender, as female gender seems to be related to atypical depression rather than melancholia (Angst et al., 2002).

We therefore studied the effect of 24 h of TSD in patients with depression. Specifically we aimed to investigate changes in the level of GLX and GLN. In an exploratory way, we also describe the other metabolites which are simultaneously assessed with 1H MR spectroscopy.

2. Materials and methods

Thirteen inpatients (eight men, five women, mean ± SD age 47.5 ± 11.8 years (31–66 years)) with MDE (ICD-classifications F32.1, F32.2, F33.1 and F33.2) scheduled for therapeutic TSD were enrolled in this study. The characteristics of these patients are given in Table 1. The patients were recruited from the psychiatric clinic of the Max-Planck-Institute of Psychiatry, Munich and have to be regarded as more difficult to treat, therefore requiring the specialized setting of this institution. Also the willingness to consent to TSD and to participate in a research project may imply that the results of the study cannot be generalized due to these biases.

The study was approved by the Ethics committee of the University of Munich and was performed according to the Declaration of Helsinki. Written informed consent has been obtained from each participant before enrolment into the study.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Diagnosis</th>
<th>HRDS-baseline</th>
<th>Medication</th>
<th>Response</th>
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<tbody>
<tr>
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<td>42</td>
<td>Mirtazapine</td>
<td></td>
</tr>
<tr>
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<td>33</td>
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<td>Mirtazapine, paroxetine</td>
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<tr>
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<td>rec. MDD</td>
<td>27</td>
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</tr>
<tr>
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<td>36</td>
<td>Citalopram, trimipramine, lorazepam</td>
<td>+</td>
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<tr>
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<td>38</td>
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<tr>
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<tr>
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<td>28</td>
<td>Mirtazapine</td>
<td></td>
</tr>
</tbody>
</table>

Response is determined as at least 50% reduction of HAMD-21 score.
Patients were assessed with MR spectroscopy before and after 24 h of TSD. All patients were rated before and after TSD using the 21 item Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960). All subjects were examined between 8.00 and 9.00 h on two consecutive days with 24 h TSD in between. Proton magnetic-resonance spectroscopy (\(^{1}H\) MRS) was performed at 1.5 T (Signa Horizon EchoSpeed (n = 11, eight male and three female patients) and Signa EchoSpeed Plus Excite II (n = 4, two male and two female patients), GE Medical Systems, Milwaukee, WI, USA) using a highly standardized acquisition technique (probe-p) and a PRESS sequence (TR/TE = 2000 or 4000/35 ms 128 averages). Two regions of interest were examined: (1) the left dorsolateral prefrontal cortex (DLPFC) and (2) the parieto-occipital cortex (POC) with TR = 4000 for the latter. Voxel sizes were aimed for consistency in anatomical landmarks around 5.8 ml for DLPFC and around 17.5 ml for POC. Concentrations of total creatine (Cr), choline-containing compounds (Cho), myo-inositol (mI), N-acetylaspartate (NAA), unresolved (GLX) and resolved GLN were estimated using LCModel software, and expressed as institutional units. The \(^{1}H\) MRS of the POC of one patient was done by using a TR of 2000 ms at both pre- and post-TSD.

3. Statistical analysis

As the main variables of interest were related to GLN metabolism, we chose GLX and GLN as the primary variables and performed an ANCOVA separately for these variables. We repeated the analysis with either gender or vegetative melancholic features (VM) (see below) as factor.

There is evidence that gender and the specific vegetative items of melancholia, i.e. reduced sleep by early morning awakening and weight loss differentiate groups of depressed patients (see Section 1). We used vegetative features given in the HAMD-21 to define a group of patients with vegetative melancholic signs (VM). This definition is based on item 6 (early awakening), item 12 (loss of appetite) and item 16 (loss of weight), in accordance with earlier studies (Overall and Rhoades, 1982; Murck et al., 2005a,b). To meet the definition of vegetative melancholia (VM) at least one of the three items had to show a maximal score of 2. We then used the resulting factor to assess the interaction of the changes due to TSD and VM and gender. In the case of a relevant interaction (p ≤ 0.1) we split the groups accordingly and performed an ANOVA in the split group. The same procedure was repeated with “gender” as a factor.

A significance level of alpha <0.05 was regarded as being significant, a level of alpha ≤0.1 was regarded as a trend. If not otherwise specified all values are given as mean ± SEM. We did not correct for multiple comparisons, as these corrections assume independence of the comparisons. This is clearly not the case here: GLN concentration contributes to GLX, therefore a correlation between these parameters exists per definitionem. The split of the group on the basis of either gender or VM are also not independent, as there is evidence that in particular sleep disturbances and weight changes depend on gender. The repetition of these analyses has therefore to be considered as mutually supportive in case the outcome points into the same direction. This procedure, however, prevents an interpretation of the analysis as confirmatory, but can only be interpreted of being either supportive or inconsistent with existing data. In particular, the overlap of both factors prevents a determination on which of them specifically is responsible for the outcome.

4. Results

4.1. Parieto-occipital cortex (POC)

No changes following TSD could be observed, with or without including the factors gender and VM into the analysis.

4.2. Dorsolateral prefrontal cortex (DLPC)

For the prefrontal cortex for GLX no significant treatment effect occurred (F(12,1) = 0.07, p = 0.80). No VM x treatment interaction existed for GLX (F = 0.64; p = 0.44). GLN did not show a change (F(12,1) = 0.51, p = 0.49) in the overall group.

Including gender as a factor in the ANCOVA revealed a trend for treatment x gender interaction for GLX (F = 3.2; p = 0.1) and a significant treatment x gender interaction for GLN (F = 4.47; p = 0.030). Splitting the group according to gender revealed a significant increase in GLX (4.35 ± 0.87 at baseline vs. 4.99 ± 0.88 after TSD; F = 8.47, p = 0.023, n = 8) and GLN (0.85 ± 0.60 at baseline vs. 1.79 ± 0.64 after TSD; F = 39.09, p = 0.001, n = 8) for male subjects. For the female patients neither GLX nor GLN showed a change.

A trend for an interaction with VM was revealed (F = 3.15, p = 0.10). This led us to split the groups into patients with or without VM. A significant increase of GLN in the VM group (0.78 ± 0.60 at baseline vs. 1.50 ± 0.76 after TSD; F = 11.25, p = 0.010, n = 9) (Fig. 1), but no significant change in the non-VM group occurred (n = 4). For GLX only a numeric increase was revealed in the VM group (4.39 ± 0.88 at baseline vs. 4.72 ± 1.29, n.s.).

4.3. Further parameters

NAA and inositol did not show any changes due to TSD and no interaction with VM or gender. The total creatine signal increased (2.80 ± 0.40 at baseline vs. 3.11 ± 0.34 after TSD, F = 7.18; p = 0.032) without a difference between patients with or without VM or between gender. The choline signal similarly were increased in the overall group (0.72 ± 0.11 at baseline vs. 0.77 ± 0.12 after TSD, F = 7.52, p = 0.029) without interaction.
For the interpretation of the data it is important to note, that the variables VM and gender are not independent in our population: From the eight males seven met the criteria of VM, whereas only two of the five female patients did so (Chi-square: $p = 0.071$), i.e. males are more likely to demonstrate melancholic vegetative characteristics than females.

4.4. Relation to clinical change

The overall group showed a strong and statistically highly significant reduction in the HAMD-21 score (28.2 ± 7.2 before vs. 16.1 ± 8.3 after TSD, $p < 0.01$) and the item 1 (depression, 2.6 ± 0.8 before vs. 1.9 ± 1.4 after TSD, $p < 0.01$). This translates into a reduction of the HAMD-21 of −43.9 ± 7.7%; range: −9% to −86% from pre-TSD and a reduction of the HAMD item 1 of 37.2 ± 10.9% (range 0% to −100%).

In the total group, there was a relation between the percentage change of the HAMD-21 and the percentage change of GLN (Pearson correlation: −0.47), which, did not reach significance level ($p = 0.127$). However, if just the percentage change of item 1 (severity of depression) is taken into account, a significant correlation of the reduction of item 1 and the increase in GLN is revealed (Pearson correlation = −0.59, $p = 0.042$), i.e. the higher the rise in GLN the better the clinical outcome. None of the other metabolites were significantly related to the clinical change.

5. Discussion

This is the first study to examine the change in brain metabolites, as measured with NMR spectroscopy, before and after TSD in patients with major depression. Our particular interest was the changes related to GLX and GLN. We could not demonstrate a significant change in the total group of patients. We revealed, however an preliminary evidence for the interaction of the changes with gender and/or VM: GLX and GLN levels were significantly increased after therapeutic-sleep deprivation in the dorsolateral prefrontal cortex in male patients. GLN showed a significant increase in patients with vegetative features of melancholia after TSD, whereas GLX was only numerically increased. The change in GLN concentration was correlated to a reduction of depressed mood, as measured with the HAMD item 1. Due to the known overlap between groups of patients determined by the presence or absence of VM on the one hand and gender on the other hand, our study cannot clarify which of these factors is predictive for a beneficial outcome with TSD. Larger scale clinical observations on this issue are required.

5.1. Accuracy of measurements

As we used an MRI apparatus with only 1.5 T the accuracy of the measurements has to be acknowledged. This is in particular the case, when metabolites, which occur only in a small concentration, like GLX or its elements, are reported. We confine the estimate of the accuracy here to the area, where the significant changes were observed, i.e. the prefrontal cortex. Further we focus on GLX and its major component GLN, as this is of major interest in this respect, because GLN has demonstrated the most consistent change in our earlier study (Murck et al., 2002). The most commonly reported measure for the accuracy of single metabolite fits is the lower Rao Cramer bound reflecting the minimum standard deviation in a percentage value from the mean of the measure. For this GLX showed standard deviations for the fitting procedure of 10–50% (mean: 23.2) in the frontal cortex. However, only one measurement showed a higher inaccuracy than 35%. This means that the measurements meet the generally accepted standards (Bender et al., 2005). If the relative measures are transformed into errors of absolute figures, the standard deviation of the fitting error for GLX has a value of 1.02 (range: 0.72–1.33), which is bigger than the change due to TSD, we described as statistically significant in male patients which was 0.69. For GLN the relative difference did not lead to reasonable values, as some had a measure of zero, which let the relative standard deviation undefined. The absolute standard deviation of the fit was 0.77 (range: 0–0.99). In absolute terms this value is close to the change of GLN in the frontal cortex in patients with VM (which was 0.72) and smaller than the difference for male patients (0.94). Overall, the low field strength remains a limitation of our study.

5.2. Comparison to related interventions and discussion of factors

Similar changes have been reported after ECT (Michael et al., 2003a; Pfleiderer et al., 2003) and ketamine administration (Rowland et al., 2005) both of which share the rapid onset of action with therapeutic-sleep deprivation. An interaction with gender or melancholia has not been reported in these studies.
There are some earlier findings for the involvement of the studied factors on the effects of sleep deprivation. For gender two studies in healthy subjects report an interaction of gender with physiological outcome variables (Armitage et al., 2001; Corsi-Cabrera et al., 2003). For VM indirect evidence for an interaction exists: the presence of the dexamethasone non-suppression, i.e. an increased hypothalamus–pituitary–adrenocortical (HPA) axis activity, may predict response to sleep deprivation (King et al., 1982). An increased HPA-axis activity seems to be related to shortened sleep and loss of weight (Künzel et al., 2003, also see Section 1). Vice versa a predictor on non-response to TSD is daytime sleepiness (Bouhuys et al., 1995), which defines atypical depression and may as such be correlated with a low HPA-axis activity in depressed patients (Levitan et al., 2002). It may therefore be the case that the presence of VM is a surrogate for an increased HPA-axis activity. The latter might be the biologically relevant predicting factor.

The observed increase in GLN in particular in the prefrontal cortex by TSD is in line with finding of an increase in activity of glutamine synthetase after sleep deprivation (in this case REM-sleep deprivation) (Bettendorff et al., 1996). It is of particular interest that this enzyme is regulated by corticosteroids in the brain (Arcuri et al., 1995; Huang and O’Banion, 1998; Laping et al., 1994). Therefore, the observed increase of GLN in the frontal cortex in those patients suggestive for a HPA-axis dysfunction on the basis of a GR resistance (male gender and/or with VM) could be the results of a sensitization of GRs by sleep deprivation. This raises also the possibility that his enzyme may be a useful target for antidepressive compounds. It is of importance to note that GLN is the main precursor of GABA synthesis (Petroff and Rothman, 1998), which links an increase of GLN to a similar change in GABAergic neurotransmission. In this sense our results are also in line with observations of an increase in GABA concentration after successful antidepressant therapy (Krystal et al., 2002).

In conclusion, this study supports the notion of a link between in increase in glutaminergic metabolites in prefrontal brain areas and rapid antidepressive manipulations, such as total sleep deprivation. We provide preliminary data that GLX increase mainly relates to GLN increase, but dedicated spectroscopic analysis at high field is required to confirm this proposition. The study also highlights the importance to differentiate patient populations with depression. Both gender and the presence of vegetative melancholic features could be of influence, but further work is needed to determine their importance for the clinical response.

Conflict of interest

There are no conflicts of interest.

Role of funding source

There was no external funding.

Author’s contribution

H. Murck, A. Steiger and D. Auer designed the study and wrote the protocol. H. Murck managed the literature searches and analyses. M. Schubert and D. Auer performed and analyzed the NMR spectroscopy. D. Schmid and P. Schüssler performed the recruitment and psychopathological assessment of the patients. H. Murck, M. Schubert and D. Auer undertook the statistical analysis, and H. Murck wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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