Effects of repetitive transcranial magnetic stimulation (rTMS) on panic attacks induced by cholecystokinin-tetrapeptide (CCK-4)

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Abstract
Low-frequency (LF) rTMS shows beneficial effects in patients with depression and anxiety disorders. To explore its anxiolytic properties we investigated the effects of rTMS on experimentally induced panic attacks. Eleven healthy subjects underwent 1 Hz rTMS or sham rTMS over the right dorsolateral prefrontal cortex in a randomized cross-over protocol. Panic induction with 50 µg CCK-4 was carried out immediately after rTMS. Response to CCK-4 was assessed using the Acute Panic Inventory and the Panic Symptom Scale and measurements of heart rate, plasma ACTH and cortisol. All subjects reported a marked panic response following CCK-4 administration after both real and sham rTMS. Moreover, injection of CCK-4 induced a marked increase in heart rate, cortisol and ACTH concentrations. However, ANOVA showed no significant differences in any of the measures between both conditions. In contrast to the effects of pretreatment with alprazolam on CCK-4-induced panic in healthy subjects LF rTMS does not affect CCK-4-induced panic and cortisol or ACTH release.

Introduction
During recent years repetitive transcranial magnetic stimulation (rTMS) has been developed as a powerful research tool in cortex neurophysiology (Kobayashi and Pascual-Leone, 2003) and a potential therapeutic intervention in neuropsychiatry (George et al., 1999). rTMS can activate or inhibit neuronal activity within the cerebral cortex depending on the stimulation parameters applied and the effects of low-frequency (LF) rTMS defined by a stimulation rate of <1 Hz are often opposite to those of high-frequency (HF) rTMS, i.e. frequencies of >1 Hz to >20 Hz (Kobayashi and Pascual-Leone, 2003; Post et al., 1999). LF rTMS reduces corticospinal excitability in primary and supplementary motor regions for >15 min after 15–25 min of stimulation (Chen et al., 1997; Romero et al., 2002). These neurophysiological effects of LF rTMS constitute the basis of potential therapeutic application of rTMS in several neurological and psychiatric disorders (Hoffman et al., 2003). Controlled clinical trials and meta-analyses provide preliminary evidence of a moderate antidepressant efficacy of LF rTMS over the right dorsolateral prefrontal cortex (DLPFC) in patients with major depression (Klein et al., 1999). In contrast, very few studies are available that investigate the effects of LF rTMS in anxiety disorders. Pilot trials suggest that rTMS might have anxiolytic properties in post-traumatic stress disorder (PTSD). Grisaru et al. (1998) found an improvement of PTSD symptoms after treatment with LF rTMS. Similarly, McCann et al. (1998) reported a reduction of PTSD symptoms after treatment with LF rTMS. A more recent study in PTSD patients, however, suggested that HF rTMS over the right DLPFC is more effective than LF rTMS (Cohen et al., 2004). In panic disorder, only case reports are thus far available. Garcia-Toro and colleagues reported on three patients suffering from panic disorder who mildly improved after LF rTMS of the right DLPFC (Garcia-Toro et al., 2002). Another case report showed a marked improvement of both anxiety and occurrence of panic attacks after LF rTMS of the right DLPFC as well as a reduction of panic symptoms experimentally induced by cholecystokinin tetrapeptide (CCK-4) and the CCK-4-
induced ACTH and cortisol release (Zwanzger et al., 2002).

Experimental panic induction with CCK-4 provides a suitable model for the evaluation of antipanic effects in novel treatment strategies (Bradwejn and Koszycki, 2001). CCK-4 induces panic attacks in both patients with panic disorder and healthy volunteers (Bradwejn and Koszycki, 2001). These experimentally induced panic symptoms are blocked or attenuated after treatment with state-of-the-art compounds for panic and anxiety. Several studies have shown that anxiolytics, which are widely used for the treatment of panic disorder lead to a reduction of panic experimentally induced with CCK-4 (Bradwejn and Koszycki, 1994; Shlik et al., 1997). Moreover, the administration of anxiolytics such as the benzodiazepine alprazolam ameliorates CCK-4-induced panic attacks in healthy volunteers (Zwanzger et al., 2003). A single-dose of 1 mg alprazolam prior to CCK-4 administration resulted in a marked attenuation of panic symptoms and ACTH and cortisol release induced by CCK-4. Therefore, experimental panic induction with CCK-4 can be used as a suitable and valid paradigm to investigate putative antipanic properties of new compounds or other therapeutic strategies. Since dose-dependently CCK-4 leads to panic attacks both in patients with panic disorder and healthy volunteers antipanic effects can also be investigated in healthy volunteers.

To experimentally investigate the anxiolytic and antipanic properties of LF rTMS we applied essentially the same CCK-4 challenge protocol as in our previous study. We replaced pretreatment with a single dose of 1 mg alprazolam by an extended LF rTMS treatment for 30 min prior to CCK-4 challenge (Zwanzger et al., 2003).

Patients and methods

Healthy subjects were screened for life-time appearances of severe illnesses by a structured interview for somatic disorders and the 12-item S-CIDI-M (Wittchen et al., 1998) questionnaire for psychiatric disorders by an experienced psychiatrist. Subjects with a current severe somatic illness or a life-time appearance of any psychiatric disorder were excluded. Eleven healthy subjects (5 male, 6 female; age 26 ± 1 years) were included. All subjects were medication free and gave their written informed consent for participation after the procedure had been fully explained. The study was approved by the local ethical review board and carried out in accordance with the Declaration of Helsinki 1975. Subjects were treated with 1 Hz real or sham rTMS in two separate sessions with a 7-d interval according to a previous design (Zwanzger et al., 2003). The sequence of the sessions was randomized and counterbalanced across subjects. Real rTMS was administered over the right DLPFC [120% intensity related to the individual resting motor threshold (RMT), 1800 stimuli, one train, 30 min] using a Magstim Rapid Stimulator (Magstim Co. Ltd, Whitland, UK) with a 70-mm figure-of-eight shaped coil. Sham stimulation was performed using a coil magnetically shielded with 2 mm of mu-metal at 20% of RMT at otherwise identical parameters as used for real rTMS. The individual RMT was determined for the left abductor pollicis brevis muscle (APBM) on a separate day prior to the first CCK-4 challenge and defined as the minimum stimulus intensity that produces a liminal motor-evoked potential (>50 µV in at least 50% of 10 trials). The position of the right DLPFC was marked 5 cm anterior in a parasagittal line to the scalp location for optimal APBM stimulation according to the standard protocol used in the majority of clinical trials investigating prefrontal rTMS as a therapeutic intervention (Herwig et al., 2001).

Experimental panic induction with 50 µg CCK-4 (Clinalfa, Läufelfingen, Switzerland) was performed immediately after treatment with real or sham rTMS respectively.

Subjects were instructed to fast 10 h prior to the CCK-4 challenge. On the day of the CCK-4 challenge an intravenous catheter was inserted in a forearm vein at 09:00 hours. Subjects sat in a supine position in a comfortable chair in a soundproof room. Through-the-wall blood drawings were performed using a long catheter running through a soundproof lock to an adjacent laboratory. To control for all extraneous interference or experimenter bias the subjects were alone in the examination room throughout the entire procedure. Communication was performed via an intercom system. rTMS treatment commenced at 09:30 hours. At 10:00 hours, 50 µg CCK-4 (Clinalfa) was administered intravenously in a bolus injection.

Panic symptoms were assessed using the Acute Panic Inventory (API) and a DSM-IV derived Panic Symptom Scale (PSS) at baseline and 5 min after CCK-4 injection. Blood samples were taken prior to rTMS treatment (baseline), 1 min prior to CCK-4 injection, and after 5, 10, 20, 30, and 60 min. The samples were placed on ice and stored at –80 °C after immediate plasma separation. Cortisol was quantified using a commercial radioimmunoassay (RIA) (Cortisol-RIA; Diagnostic Products Corporation, Biermann, Bad Nauheim, Germany). The lower detection limit was 8.28 nmol/l. Intra- and inter-assay coefficients of
variation (CV) were 2.4% and 6.4% respectively. The maximum heart rate was recorded with a Datex-Ohmeda light monitor (Datex-Ohmeda, Finland).

Results were analysed applying a one-way analysis of variance (ANOVA). Changes of ACTH and cortisol stimulation after CCK-4 injection were calculated according to the trapezoidal rule and are expressed as area under the curve (AUC). All results are given as mean ± S.E.M. and the nominal level of significance was set at \( \alpha = 0.05 \). The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

**Results**

Subjects reported a marked panic response to CCK-4 administration both after real and sham rTMS (Table 1). The mean API and PSS sum scores did not differ significantly between real and sham treatment [API: \( F(1,20) = 0.031, p = 0.863 \); PSS: \( F(1,20) = 0.011, p = 0.917 \)]. In addition, there was no significant difference in the number of reported symptoms [\( F(1,20) = 0.000, p = 1.000 \)]. CCK-4-induced panic was accompanied by a marked increase in heart rate. However, there was no difference in the maximum heart rate between both groups [\( F(1,20) = 0.041, p = 0.841 \)]. There was no significant group difference in plasma cortisol and ACTH levels either before (baseline) or after rTMS treatment prior to CCK-4 injection (pre-CCK-4) [cortisol baseline: \( F(1,20) = 0.031, p = 0.862 \); cortisol pre-CCK-4: \( F(1,20) = 0.218, p = 0.646 \); ACTH baseline: \( F(1,20) = 0.018, p = 0.893 \); ACTH pre-CCK-4: \( F(1,20) = 0.023, p = 0.828 \)]. Administration of CCK-4 induced a marked increase in both cortisol and ACTH concentrations (Table 1). However, there was also no significant difference in either cortisol and ACTH peak plasma levels (max) or cortisol and ACTH profiles (AUC) during the CCK-4 injection between both groups [cortisol max: \( F(1,20) = 0.438, p = 0.516 \); ACTH max: \( F(1,20) = 0.026, p = 0.873 \); cortisol AUC: \( F(1,20) = 0.278, p = 0.604 \); ACTH AUC: \( F(1,20) = 0.004, p = 0.952 \)].

**Discussion**

To our knowledge, this is the first study investigating effects of rTMS treatment on experimentally induced panic attacks. The main finding was that treatment with LF rTMS did not affect CCK-4-induced panic and cortisol and ACTH release in healthy volunteers. In a previous study including the same CCK-4 challenge protocol a single dose of 1 mg alprazolam reduced both panic and cortisol/ACTH release in healthy subjects (Zwanzger et al., 2003). Very recently, reduction

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**Table 1.** Maximum Acute Panic Inventory (API) and Panic Symptom Scale (PSS) scores (mean ± S.E.M.) during CCK-4-induced panic after verum and placebo rTMS; number of reported symptoms; maximum heart rate (HR max); plasma cortisol and ACTH concentrations before rTMS (baseline) and after rTMS/prior to CCK-4 injection (pre-CCK-4); hormone concentrations during the CCK-4 challenge: peak levels of cortisol and ACTH concentrations (max) and change in cortisol and ACTH levels reflected by areas under the curve (AUC) in both groups (mean ± S.E.M.)

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<tr>
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<th>Real rTMS</th>
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<th>Sham rTMS</th>
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<td>Mean ± S.E.M.</td>
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<td>Mean ± S.E.M.</td>
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<td>Clinical measures</td>
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<td>API</td>
<td>25.2 ± 3.9</td>
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<td>27.0 ± 3.2</td>
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<td>PSS</td>
<td>18.7 ± 3.3</td>
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<td>18.3 ± 2.8</td>
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<tr>
<td>Number of reported symptoms</td>
<td>9.3 ± 1.41</td>
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<td>9.3 ± 1.11</td>
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<tr>
<td>HR max (beats/min)</td>
<td>113 ± 3.6</td>
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<td>114 ± 3.4</td>
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<td>Cortisol plasma levels</td>
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<td>Cortisol baseline (nmol/l)</td>
<td>494 ± 118</td>
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<td>469 ± 80</td>
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<td>Cortisol pre CCK-4 (nmol/l)</td>
<td>471 ± 102</td>
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<td>413 ± 68</td>
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<tr>
<td>Cortisol max (nmol/l)</td>
<td>573 ± 113</td>
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<td>492 ± 49</td>
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<td>Cortisol AUC (nmol/l . min)</td>
<td>30 846 ± 6125</td>
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<td>27 231 ± 3173</td>
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<td>ACTH plasma levels</td>
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<td>ACTH baseline (pmol/l)</td>
<td>4.5 ± 0.7</td>
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<td>4.4 ± 0.7</td>
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<tr>
<td>ACTH pre CCK-4 (pmol/l)</td>
<td>4.9 ± 1.0</td>
<td></td>
<td>5.2 ± 1.5</td>
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<td>ACTH max (pmol/l)</td>
<td>15.1 ± 4.4</td>
<td></td>
<td>16.3 ± 6.6</td>
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<td>ACTH AUC (pmol/l . min)</td>
<td>587 ± 134</td>
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<td>603 ± 235</td>
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of CCK-4-induced panic has also been observed after a 30-min aerobic treadmill exercise in healthy subjects (Ströhle et al., 2005). Thus, the effects of a single LF rTMS session on experimentally induced panic do not reach the efficacy of a standard pharmacological intervention or even aerobic exercise in healthy subjects.

These results seem to be in contrast to the beneficial effects reported in patients with PTSD after a single session of LF rTMS (Grisaru et al., 1998) or extended LF rTMS treatment (McCann et al., 1998). Moreover, the results are contrary to the previously reported improvement of panic disorder and CCK-4-induced panic in a patient with panic disorder (Zwanzger et al., 2002). However, it has to be considered that in the latter two case studies rTMS treatment was administered for ≥10 d, while in the current study only a single rTMS session was applied. Therefore, treatment duration may have been too short to overrule the effect of a strong panicogen like CCK-4. Another possible explanation for the lack of panic amelioration after rTMS treatment may be that an hypothesized inhibitory action of LF rTMS on the prefrontal cortex would be more likely to improve general anxiety and its cognitive appraisal than panic symptoms, which are mainly generated in areas of the brainstem (Gorman et al., 2000).

Our results add to previous findings of absent or mild effects of rTMS on mood and anxiety in healthy subjects (Schutter et al., 2001). Whereas single reports in healthy subjects suggested anxiolytic-like effects after LF rTMS (Schutter et al., 2001), other studies did not show any changes in mood or anxiety scores after rTMS (Grisaru et al., 2001; Jenkins et al., 2002; Mosimann et al., 2000; Padberg et al., 2001).

No effects of rTMS treatment were observed on hypothalamic–pituitary–adrenal (HPA) axis activity in the present study. Although the relationship between severity of CCK-4-induced panic symptoms and HPA stimulation is discussed controversially there is some evidence that the CCK-4-induced HPA stimulation might be associated with the severity of experimentally induced panic. It has been shown that a severe panic response to CCK-4 is accompanied by a strong HPA axis activation, whereas HPA stimulation in non-panickers is far less pronounced (Koszycki et al., 1998; Ströhle et al., 2000). Moreover, reduction of CCK-4-induced panic after anxiolytic treatment with alprazolam is also accompanied by an attenuated HPA response (Zwanzger et al., 2003). The study design, however, did not allow investigation of the effects of LF rTMS on HPA axis activity and such effects cannot be excluded. Findings from animal experiments indeed suggest that rTMS attenuates the neuroendocrine stress response in rats (Keck et al., 2000) and the question whether rTMS exerts effects on HPA axis activity needs to be addressed in future studies.

In conclusion, despite preliminary clinical evidence of beneficial effects of LF rTMS in patients with anxiety disorders LF rTMS appears not to be effective for the amelioration of CCK-4-induced panic in healthy volunteers. Future studies should investigate the effects of extended rTMS treatment protocols in patients with panic disorder and also apply experimental panic induction paradigms.

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Statement of Interest

None.

References


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