Brain Stimulation 10 (2017) 604–608

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Cortical inhibitory and excitatory function in drug-naive generalized anxiety disorder

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ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form
7 December 2016
Accepted 15 December 2016
Available online 21 December 2016

Keywords:
Motor cortex
Generalized anxiety disorder
Intracortical inhibition
Intracortical facilitation
Glutamate
GABA

ABSTRACT

Background: A growing body of evidence suggests that deficits in GABAergic inhibitory and glutamatergic excitatory neurotransmission may be involved in the core pathophysiology of generalized anxiety disorder (GAD), a disease characterized by pathological anxious worrying. The aim of the present study was to measure motor cortical excitability by paired-pulse transcranial magnetic stimulation (ppTMS) in patients with GAD.

Methods: ppTMS measurements of excitation and inhibition from bilateral motor cortices were investigated in 26 right-handed GAD patients who were drug-naïve (half of them with a comorbidity of major depressive disorder) and 35 right-handed age- and sex-matched healthy controls. Short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long-interval intracortical inhibition (LICI) were studied; evidence indicated that these are mainly mediated by GABA-A receptors, glutamate receptors, and GABA-B receptors, respectively.

Results: After correcting for multiple comparisons, GAD patients had significantly lower left ICF ($p < 0.001$, Cohen's $d = 1.348$) and right ICF ($p = 0.001$, Cohen's $d = 0.963$), but not SICI and LICI, than did healthy controls. No significant difference of the ICF values was found between GAD with and without depressive disorders. Multivariate linear regression analysis revealed that left ICF ($B = -4.990$, 95% CI $= -8.821$ to -1.039, $p = 0.007$) and group ($B = 13.179$, 95% CI $= 10.208$ to 16.149, $p = 0.001$) predicted anxiety symptoms significantly.

Conclusion: The present study provided direct evidence to support that generalized anxiety disorder is characterized by impaired intra-cortical facilitation of motor cortex, suggesting glutamate-related excitatory dysfunction may play a key role in pathological anxiety.

1. Introduction

Generalized anxiety disorder (GAD) is characterized by excessive anxiety and worry, which is uncontrollable and apprehensive expectation about a number of events or activities for a long period of time. GAD tends to be chronic and is associated with impaired health-related quality of life and substantial disability [1]. The core pathological mechanisms underlying anxiety disorders have yet to be fully understood; however, a growing body of evidence suggests that GABA and glutamate may be involved in the mechanisms.

The gamma-aminobutyric acid (GABA) system is believed to play a key role in the pathophysiology of GAD [2]. GABA is the major inhibitory neurotransmitter in the brain and acts at inhibitory

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http://dx.doi.org/10.1016/j.brs.2016.12.007
1935-861X/

synapses by binding to specific receptors, including GABA-A and GABA-B receptors [3]. Benzodiazepines, as one of the most commonly-prescribed drugs for GAD, enhance the effect of the neurotransmitter GABA at the GABA-A receptor [4]; however, long-term treatment by benzodiazepines is inadequate because of the risk of benzodiazepine dependence [5]. Clinically, the role of GABA-B receptors in the pathophysiology of GAD seems to have been explored less than the role of GABA-A [6]. Nonetheless, preclinical evidence has shown that GABA-B receptors play a role in the pathophysiology of anxiety disorders [7]. Other drugs targeting the GABA system might be effective for GAD. For example, pregabalin, a GABA analog, could be effective in treating GAD, and it has a lower tendency for abuse and dependence [8]. Results for GABA-B agonists have been somewhat inconsistent but also demonstrated potential in the treatment of anxiety disorders, such as panic disorders and anxiety associated with alcohol withdrawal [7]. GABAergic inhibition is essential for neuronal information processing through a close interaction with glutamatergic pyramidal neurons [3].

Glutamate-mediated neurotransmission has also been implicated in anxiety and related disorders [9]. Glutamate is ubiquitous and is the major excitatory neurotransmitter in the brain. Neural activity and adaption are critically shaped by dynamic interaction and the balance of excitation and inhibition [10,11]. However, glutamate excito-toxicity has been associated with exposure to severe stress. For example, excessive glutamate release and uptake have been found in the mood-regulating brain regions such as the frontal cortex and hippocampus of rats exposed to various forms of stress [12,13]. Preclinical evidence has demonstrated that medications which alter glutamate transmission have the potential to treat anxiety [9]. Preliminary clinical drug trials using compounds which act on the glutamate system have also provided support for the role of glutamate abnormalities in the pathophysiology of GAD [14,15].

In order to study GABA-related inhibitory and glutamate-related excitatory functions of the brain, the present study utilized paired-pulse repetitive transcranial magnetic stimulation (ppTMS) in patients with GAD. ppTMS is a non-invasive technique that manipulates the strength and the stimulus intervals between two pulses in order to measure cortical inhibition and excitation in humans [16,17]. ppTMS could be used to examine at least two different cortico-cortico inhibitory processes in the human motor cortex that are medicated by different subtypes of GABAergic receptors: short-interval cortical inhibition (SICI) and long-interval cortical inhibition (LICI) [18]. There is evidence suggesting that SICI is mainly mediated by GABA-A mediated inhibitory interneurons [19,20]. Different from SICI, GABA-B receptors are thought to play an important role in mediating LICI [17]. On the other hand, ppTMS could also be used to examine a cortico-cortico excitatory process, intracortical facilitation (ICF) [16,21]. which is critically mediated by glutamatergic neurotransmission [22].

Given ppTMS's high sensitivity in detecting subtle deficits in cortical inhibition and excitation, ppTMS has evolved as an excellent tool to study abnormalities in cortical excitability associated with major neuropsychiatric disorders [23–25]. To date, there is still a lack of ppTMS studies specifically focused on GAD. Here, we used ppTMS to study cortical excitatory and inhibitory function in patients with GAD.

2. Methods and materials

2.1. Subjects

This study included 26 right-handed patients (mean age, 42.0 ± 9.7; age range from 23 to 60; 13 males) with a DSM-IV diagnosis of GAD confirmed by the Mini International Neuropsychiatric Interview (MINI) based on the *Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)* criteria (American Psychiatric Association, 2000) and 35 right-handed healthy subjects (mean age, 41.0 ± 10.6; age range from 23 to 59; 15 males) who were free of major medical and neurological illnesses or a history of alcohol or substance abuse. In addition, all healthy control subjects were free from a diagnosis of psychiatric disorder as determined by the MINI. Handedness was confirmed using the Edinburgh Handedness Inventory [26]. All patients with GAD were drug-naïve. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Review Committee of the Taipei Veterans' General Hospital. Informed consent was completed by all participants.

2.2. Clinical evaluations

Patients and healthy control subjects underwent a detailed psychiatric and medical history-taking, a diagnostic interview by the MINI, and symptom ratings for anxiety symptoms using the Hamilton Anxiety Rating Scale (HARS) [27].

2.3. Paired-pulse TMS (ppTMS) procedures

Surface electromyography (EMG) recordings were made from the bilateral abductor pollicis brevis (APB) muscles, through Ag-AgCl electrodes with the active electrode over the belly of the APB. Subjects kept the hand muscles relaxed throughout the experiment. TMS was applied to the hand area of the left and right motor cortices with a 70-mm figure-of-eight coil connected to Magstim 200 magnetic stimulators (Magstim company, Whitland, UK) in two separated sessions. Two stimulators were connected to the same coil through a bi-stim module (Magstim). The coil was placed at the optimal position for eliciting MEPs from the left or right APB muscle and was held tangentially on the head with the coil handle pointing backward, about 45° away from the mid-sagittal line.

The resting motor threshold (rMT, expressed as the percentage of maximum stimulator output) was defined as the lowest intensity which produced an MEP of $\geq$ 50 μV in 5 of 10 trials in the relaxed APB muscle [16]. Intra-cortical facilitation (ICF) and short-interval intra-cortical inhibition (SICI) were tested with a subthreshold conditioning stimulus (CS) at 80% of rMT preceding a suprathreshold testing stimulus (TS) at 120% of rMT [16]. SICI results in attenuation of the amplitude of motor-evoked potential (MEP) when a subthreshold pulse precedes a suprathreshold pulse by 1–5 ms (ms) [16], while ICF results in facilitation of the MEP response when a subthreshold pulse precedes a test pulse by 8–30 m [16,21]. In the present study, interstimulus intervals (ISIs) between CS and TS of 2, 5, 10 and 20 m were tested for measuring SICI (CS2 and CS5) and ICF (CS10 and CS20), respectively.

Long-interval intra-cortical inhibition (LICI) was tested with suprathreshold CS (i.e., 120% of rMT) followed by TS at 120% of rMT at ISIs of 100 and 200 m (CS100 and CS200) [17]. LICI results in attenuation of the MEP amplitude when a suprathreshold conditioning pulse precedes a suprathreshold test pulse by 100–200 m [17]. Each session for one side of the motor cortex consisted of eight trials of TS alone (unconditioned responses) and eight trials for each of the six conditioned stimuli (i.e., CS2, CS5, CS10, CS20, CS100, and CS200). In total, 56 trials were held and each of the 56 trials was presented every eight seconds in random order. All the trials and their stimulus intensities were programmed and automatically adjusted by Signal (Version 6.02, Cambridge Electronic Design Ltd. Cambridge, England) through a control cable. The order of the two sessions was counterbalanced to avoid order effects. The conditioned MEPs were calculated as the ratio of the mean MEP