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Neuropeptide Y as a possible homeostatic element for changes in cortical excitability induced by repetitive transcranial magnetic stimulation

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ABSTRACT

Background: Repetitive transcranial magnetic stimulation (rTMS) is able to modify cortical excitability. Rat rTMS studies revealed a modulation of inhibitory systems, in particular that of the parvalbumin-expressing (PV+) interneurons, when using intermittent theta-burst stimulation (iTBS).
Objective: The potential disinhibitory action of iTBS raises the questions of how neocortical circuits stabilize excitatory-inhibitory balance within a physiological range. Neuropeptide Y (NPY) appears to be one candidate.
Methods: Analysis of cortical expression of PV, NPY and vesicular glutamate transporter type 1 (vGluT1) by immunohistochemical means at the level of cell counts, mean neuropil expression and single cell pre-/postsynaptic expression, with and without intraventricular NPY-injection.
Results: Our results show that iTBS not only reduced the number of neurons with high-PV expression in a dose-dependent fashion, but also increased the cortical expression of NPY, discussed to reduce glutamatergic transmission, and this was further associated with a reduced vGluT1 expression, an indicator of glutamateric presynaptic activity. Interneurons showing a low-PV expression exhibit less presynaptic vGluT1 expression compared to those with a high-PV expression. Intraventricular application of NPY prior to iTBS prevented the iTBS-induced reduction in the number of high-PV neurons, the reduction in tissue vGluT1 level and that presynaptic to high-PV cells.
Conclusions: We conclude that NPY, possibly via a global but also slow homeostatic control of glutamatergic transmission, modulates the strength and direction of the iTBS effects, likely preventing pathological imbalance of excitatory and inhibitory cortical activity but still allowing enough disinhibition beneficial for plastic changes as during learning.

Introduction

A rich variety of inhibitory interneurons controls the proper function of cortical networks. This includes not only limitation of excitatory activity via a well-tuned excitatory-inhibitory balance, but also coordination of cell assemblies in space and time [1]. On the other hand, state-dependent modulation of the inhibitory status appears to be essential to enable memory-related synaptic plasticity and activity-driven neuronal network development [2,3]. Transient changes in the balance between excitatory and inhibitory activity thus play an important role in physiological processes but a persistent imbalance due to malfunction of inhibitory neurons appears to be the reason of many neurological and psychiatric diseases [4–7], in particular with respect to the fast-spiking, parvalbumin-expressing (PV) interneurons [8–10]. Repetitive transcranial magnetic stimulation (rTMS) has been shown to modulate cortical excitability and to promote learning [11,12], potentially via transient changes in the excitation-inhibition balance [13–16]. These procedures thus offer therapeutic options to correct imbalanced activity and to induce targeted neuronal plasticity via concomitant training procedures [13], but could possibly induce a pathological imbalance of systems.

Abbreviations: ANOVA, analysis of variance; FSI, fast-spiking interneurons; iTBS, intermittent theta-burst stimulation; LTD, long-term depression; LTP, long-term potentiation; MANOVA, multiple analysis of variance; MB, methylene blue; NPY, neuropeptide Y; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PNNs, perineuronal nets; PV, parvalbumin; ROI, region of interest; rTMS, repetitive transcranial magnetic stimulation; vGluT1, vesicular glutamate transporter type 1.

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Our previous studies in rat TMS models showed that intermittent theta-burst stimulation (iTBS, [17]) is able to strongly reduce the number of neurons with high expression of the calcium-binding protein parvalbumin (PV, [18]) which is found primarily in fast-spiking inhibitory interneurons (FSI, [19]). A likely cortical disinhibition was indicated by increased cortical sensory responses [20] and increased excitatory cortical network activity in vitro [21]. Network disinhibition risks cortical over-excitation which may be prevented by adjunct homeostatic mechanisms modulating glutamatergic transmission. Animal studies revealed increased neuropeptide Y (NPY) tissue levels after strong activation such as by seizures [22–24]. NPY has the potential to modulate excitatory synaptic transmission by regulating presynaptic glutamate release via Y2-type receptors [25]. Therefore, the present study aimed to investigate how iTBS, in addition to decreasing PV expression in rat neocortex, regulates the expression of NPY and that of the vesicular glutamate transporter (vGluT1) which is found in excitatory synaptic terminals of cortical origin [26]. We further studied if artificial enhancement of the cortical NPY level prior to iTBS, achieved via intraventricular injection, can alter the iTBS effect, e.g. the reduction in the number of cells with strong PV expression. Finally, we studied the relationship between PV expression in individual interneurons (high-vs low-PV, see Ref. [27]) and the amount of vGluT1 expression in terminals presynaptic to PV-type interneurons for the different experimental conditions (sham vs verum iTBS and NPY vs vehicle injection, see Fig. 1).

Materials and methods

Animals and study design

The study was composed of three parts: 1) the action of iTBS on cortical PV, NPY and vGluT1 expression, and quantification of vGluT1 expression presynaptic to individual PV-type interneurons (5–6 animals per group), 2) the same analyses subsequent to an intraventricular NPY (or vehicle) injection 60 min prior to iTBS (6 animals per group), 3) quantification of presynaptic vGluT1 on PV-type interneurons at a different age independent from iTBS to see if physiological processes like maturation of these interneurons during early cortical maturation (critical period) also affects the relationship between PV expression and glutamatergic input (6 animals per age-group, using material of a former study [28]).

Generally, adult (about 3 month) male Sprague Dawley rats (Elevage Janvier) were used for this study (parts 1 and 2). Only study part 3 included rats aged between 28 and 60 days. All rats were housed under standard laboratory conditions (12 h light/dark with light-on at 7am) in groups of maximum three rats per cage with free access to food pellets and water. All experimental procedures had been approved by the ethics section of the local government (LANUV, Az. 87–51.04.2010.A097) and are in compliance with the guidelines of the animal welfare laws in Germany and the EU.

Anaesthesia, surgical procedures and NPY application

Rats were sedated with urethane (20%; 1.5 g per kg body weight, Sigma-Aldrich) about 70 min prior to surgical procedures and iTBS application. In case of surgical procedures needed to intracranially inject NPY, ketamine hydrochloride (10%, 60 mg per kg body weight, i.m., CP-Pharma, Burgdorf, Germany) was added for analgesia. After the rat had been fixed by the aid of a stereotaxic frame and after the scalp was opened by a sagittal cut, a cranial hole of 2 mm in diameter was drilled and a cannula connected to a 50 μl Hamilton syringe was lowered to the left lateral ventricle using the following coordinates (according to rat brain atlas by Paxinos and Watson [29]): 0.5 posterior and 1.6 lateral to the bregma and to a depth of about 4 mm. Either 20 μl NPY (24 nMol in saline ≡ 2 μg NPY; Sigma-Aldrich) or methylene blue (MB in saline, Sigma-Aldrich) as a vehicle and to check the spatial range of diffusion was injected at a rate of 2 μl/min. The total amount of NPY injected is within the range tested by others to reduce hippocampal seizure activity and related neuronal apoptosis [30–32]. We decided to inject a larger volume to the lateral ventricle that was done in the hippocampal studies (2–10 μl) to force NPY diffusion also to the neocortical areas more distant to the ventricular lumen. The cannula was kept in place for further 10 min before being slowly retracted. At the end of the experiment, anaesthesia was deepened with pentobarbital-sodium (300 mg/kg bw; Narcoren; Rhone Merieux GmbH, Laupheim, Germany) before rats were perfused transcardially about 60 min after rTMS, first using cooled physiological saline containing heparin (5000 IE; Liquemin; Roche, Basel, Switzerland), followed by cooled 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS pH 7.4). For standard immunohistochemical procedure see former publications [14,18] and supplementary material.

rTMS (iTBS)

For reasons of better comparability with previous studies [14,32] rats of the study parts 1 and 2 received the iTBS application while sedated with urethane. Using a Magstim Rapid^2 (The Magstim company, Whitland, Dyfed, UK) all rats received the iTBS protocol via a figure-of-eight coil (2 × 70 mm) at a stimulation strength just subthreshold for inducing motor responses, corresponding to 23–26% of maximum machine power (21–23% are sufficient in conscious rats). Each iTBS block consisted of 20 trains of ten 50 Hz bursts (3 pulses) repeated at 5 Hz (2s On, 8s Off, 600 pulses in total, see Ref. [17]). Up to five blocks were applied at 15 min intervals. The figure-of-eight coil was centred a few millimetres above the head without mechanical contact to the skin. The coil was oriented in a way to induce an electric field of mediolateral direction, suitable to activate the long axons of the corpus callosum at the low stimulation intensity, thereby inducing transsynaptic but also antidromic activation of supragranular (L2/3) cortical neurons (for more details see Ref. [18]). In case of sham stimulation, the distance between coil and head was increased to 100 mm, thereby greatly weakening the magnetic field strength at the brain but still keeping the same acoustic scenario of sound generated by the TMS coil.

In the first study, sham-control rats were compared with those receiving between one and five iTBS blocks (600 pulses/block). In the second study, rats received three blocks of iTBS (or sham) 60 min following intraventricular NPY injection. Three blocks were chosen because we found just significant effects on PV-expressing neurons (first part of this study, as well as previous studies: [20,21,33]) and to avoid possible saturation/ceiling effects more likely with four to five iTBS blocks.

Data collection and analyses

Analysis of immunohistochemical labelling was done in rat primary motor cortex region with sections obtained from anterior (2.7–3.7 mm anterior to bregma) and posterior parts of M1 (0.5–1.5 mm anterior to bregma and about 1.5–3 mm lateral to midline). Regions of interest (ROIs) were photographed, using a Leitz Wetzlar Dialux 20 microscope (Leica, Germany) equipped with a colour video camera (Leica DFC300FX) connected to Metamorph Universal Imaging System (Visitron System, Puchheim, Germany). Cell-bodies immunoreactive to PV or NPY were counted manually while NPY and vGluT1 immunoreactivity of the neuropil

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