Carbonic Anhydrase I, II, and VI, Blood Plasma, Erythrocyte and Saliva Zinc and Copper Increase After Repetitive Transcranial Magnetic Stimulation

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Abstract: *Introduction:* Repetitive transcranial magnetic stimulation (rTMS) has been used to treat symptoms from many disorders; biochemical changes occurred with this treatment. Preliminary studies with rTMS in patients with taste and smell dysfunction improved sensory function and increased salivary carbonic anhydrase (CA) VI and erythrocyte CA I, II. To obtain more information about these changes after rTMS, we measured changes in several CA enzymes, proteins, and trace metals in their blood plasma, erythrocytes, and saliva. *Methods:* Ninety-three patients with taste and smell dysfunction were studied before and after rTMS in an open clinical trial. Before and after rTMS, we measured erythrocyte CA I, II and salivary CA VI, zinc and copper in parotid saliva, blood plasma, and erythrocytes, and appearance of novel salivary proteins by using mass spectrometry. *Results:* After rTMS, CA I, II and CA VI activity and zinc and copper in saliva, plasma, and erythrocytes increased with significant sensory benefit. Novel salivary proteins were induced at an *m*/*z* value of 21.5K with a repetitive pattern at intervals of 5K *m*/*z*. *Conclusions:* rTMS induced biochemical changes in specific enzymatic activities, trace metal concentrations, and induction of novel salivary proteins, with sensory improvement in patients with taste and smell dysfunction. Because patients with several neurologic disorders exhibit taste and smell dysfunction, including Parkinson disease, Alzheimer disease, and multiple sclerosis, and because rTMS improved their clinical symptoms, the biochemical changes we observed may be relevant not only in our patients with taste and smell dysfunction but also in patients with neurologic disorders with these sensory abnormalities.

Key Indexing Terms: Carbonic anhydrase; Zinc; Copper; Sensory changes after transcranial magnetic stimulation; Mass spectrometry.
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Investigators have used transcranial magnetic stimulation in patients with many disorders, including neurologic disorders such as stroke, epilepsy, facial palsy, dystonia, multiple sclerosis, Alzheimer disease, Parkinsonism,[1–16] and mood disorders and schizophrenia.[17,18] Associated with this treatment have been reports of changes in several neurotransmitters and neuroactive agents, including dopamine,[19–25] catecholamines and other biogenic amines,[26–30] serotonin,[31,32] gamma-aminobutyric acid (GABA),[33–35] hormones,[36–38] other chemical moieties,[39–48] and interactions among these moieties[27–29,40,46] in the brain, blood plasma, and saliva.

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Abnormalities of taste and smell function have been reported in patients with several of these neurologic conditions, including multiple sclerosis,[49–53] Alzheimer disease,[54–58] Parkinson disease,[59–62] and other neurodegenerative disorders.[63,64] In patients with Parkinson disease, smell loss has been considered the first sign of the disease,[60] the more severe the disease process the more severe the smell loss.[65] Phantosmia (the presence of olfactory distortions in the absence of any environmental odor) has also been reported in Parkinson disease.[66] Changes in several neurotransmitters and neuroactive substances known to play significant roles in taste and smell function have been reported to occur in some of these disorders. These moieties include dopamine,[67–71] catecholamines,[72–75] serotonin,[76,77] GABA,[78–87] trace metals,[88–92] adenylyl cyclases,[93–98] hormones,[99,100] other chemical moieties,[101–104] and interactions among these moieties.[76,83,85,86]

Repetitive transcranial magnetic stimulation (rTMS) has been used to treat clinical manifestations of patients with multiple sclerosis,[11–13] Alzheimer disease,[14,15] Parkinson disease,[17–20] and other neurologic conditions,[1–10] and changes in several chemical moieties in the brain, blood plasma, and saliva, which play roles in taste and smell function, have been measured with this therapy.[19–25,35–38,40] Although clinical changes in taste and smell function have not been reported after rTMS in patients with neurodegenerative disorders, other specific therapies have been reported to be successful in improving taste and/or smell dysfunction in patients with Parkinson disease by some[105,106] but not by other[107] investigators.

Phantogeusia (presence of taste distortions in the absence of food or drink), phantosmia,[108–110] and loss of taste and smell acuity have been reported in patients without other neurologic or otolaryngologic abnormalities. We previously demonstrated decreased GABA by using magnetic resonance spectroscopy in specific brain regions in some of these patients, including those with phantogeusia and/or phantosmia.[87,111] On the basis of these results, we used GABAergic drugs to treat these patients[111,112]; results indicated that this treatment increased their brain GABA[88,111] and improved their sensory abnormalities.[112] In some of these studies, we observed increased secretion of salivary carbonic anhydrase (CA) VI,[113] a zinc-containing metalloglycoprotein,[114–116] a putative taste bud and olfactory epithelial growth factor[91,92,117,118] similar to nerve growth factor.[92,118–121] CA VI secretion was previously considered a marker for both taste[91,92,118] and smell function[122] associated with correction of pathologic apoptotic changes in both taste bud[90] and olfactory epithelial structures.[109] Thus, we hypothesized that increased CA VI was associated with taste bud and olfactory epithelial stem cell stimulation.[110]

Because correction of these sensory abnormalities occurred with GABAergic drug treatment and because GABA[33–35] and other neurotransmitters had been altered with

rTMS,[19–29,39–47] we hypothesized that rTMS treatment might also correct these sensory abnormalities. In preliminary studies in a single-blind placebo-controlled fixed sequence clinical trial, rTMS corrected these sensory abnormalities.[112] These results suggested that CA VI might also change after rTMS. Thus, before and after rTMS, we measured CA VI activity and observed changes in this enzyme and in other salivary proteins. On the basis of our previous studies of salivary proteins,[115] because CA VI is a zinc containing metalloglycoprotein, and to learn more about these phenomena, we also measured salivary zinc and copper concentrations and changes in other salivary proteins. On the basis of our experience with these patients, we also hypothesized that little or no change would occur in any peripheral enzyme system. Therefore, we also measured changes in erythrocyte CA I, II and concentrations of zinc and copper in both erythrocytes and blood plasma to test this latter hypothesis.

METHODS

Ninety-three patients, aged 18 to 85 years (52 ± 2 years, mean ± SEM), 49 men, aged 29 to 74 years (51 ± 3 years) and 44 women, aged 20 to 85 years (53 ± 3 years), with phantogeusia and/or phantosmia, hyposmia (loss of smell acuity), and hypogeusia (loss of taste acuity) were studied before and after rTMS in a single-blind placebo-controlled fixed sequence clinical trial. Studies were performed consistent with a protocol approved by the institutional review board of the George Washington University Medical Center, and each patient agreed to participate in this study.

Patient symptoms persisted for 0.4 to 30 years (6.9 ± 1.5 years) before rTMS. Physical examination of the head and neck, including examination of oral and nasal cavities, was within normal limits. Neither neurologic nor psychiatric abnormalities other than taste and/or smell dysfunction was present in any patient. Anatomical magnetic resonance imaging (MRI) of the brain and electroencephalographic studies were within normal limits.

rTMS was performed with a Cadwell magnetic pulse stimulator (Kennewick, WA) monitored with a TECA TD20 (Pleasantville, NY) waveform generator, as previously briefly described.[113,114] Stimulation was applied in a fixed manner by using a single circular 5 cm (internal diameter) coil to 4 skull locations (left and right temporoparietal, occipital, and frontal). Stimulus frequency was 1 pulse given per 1 to 3 seconds for 30 to 60 seconds, with 20 pulses given at each location. Repeat stimulation was performed in all patients in whom sensory distortions decreased and/or sensory acuity increased; repetition continued (2–6 applications) until no further decrease in sensory distortions and/or increase in sensory acuity occurred.

One hour before and 1 to 2 hours after completion of rTMS, venous blood and parotid saliva were collected. Venous blood was placed into zinc-free tubes containing 100 μL of zinc-free heparin, on ice, centrifuged at 3000 rpm at 4°C, plasma removed, and stored at −20°C until assayed. Erythrocytes were washed and treated as previously described.[123] Parotid saliva was collected using a modified Lashley cup applied to Stensen duct, with maximal stimulation using reconstituted lemon juice applied to the lingual surface as previously described.[114,115] Five hundred microliters of saliva was placed in dry ice immediately after collection and stored at −60°C for measurements by surface-enhanced laser desorption/ionization time-of-flight mass analysis vide infra.

Zinc and copper were measured by atomic absorption spectrophotometry by methods previously described[123,124] using a double-beam ThermoJarrell Ash video 22 (Franklin, MA) atomic absorption spectrophotometry modified by the Maxwell Instrument Company (Salisbury, NC). Saliva protein was determined by measurement of total peptide content by using absorbance at A215-A225 and the extinction coefficient, as previously described.[114]

CA activity was measured by a modification of the method of Richli et al,[125] as previously described. CA activity is expressed as enzyme concentration per milligram of enzyme protein.

Saliva samples stored at −60°C were thawed, and 1 μL was directly spotted on an H4 Protein Chip array (prewashed with 0.1% trifluoroacetic acid in 50% aqueous acetonitrile) and their protein profile examined on a Ciphergen (Fremont, CA) PBS IIc mass analyzer. Samples were first incubated in a humid chamber for 5 to 10 minutes at room temperature, then washed with 5% aqueous acetonitrile, dried, and 1 μL matrix was added (sinapinic acid in 0.1% trifluoroacetic acid, 50% aqueous acetonitrile). Samples were allowed to dry again and subjected to SELDI-TOF analysis on the PBS IIc. Protein peaks were characterized by their apparent molecular weight [based on their mass/charge ratio (m/z)].

Following initial observation of biochemical changes after rTMS, subsequent measurements in all biological fluids were performed in a blinded manner; all samples were coded and results uncoded only after analyses were completed.

Mean ± SEM for each parameter was determined before and after rTMS. Differences were calculated for each parameter, and significance of differences was determined by parametric (differences between undifferentiated means, paired t tests, χ^2) and nonparametric (sign test) statistics; $P < 0.05$ was considered significant for all statistical analyses.

Table 2 summarizes changes in each parameter measured in the total patient group of 93 patients. Two sets of numbers are shown. One set reflects the number of patients in whom there was no change from each parameter value measured comparing after with before rTMS. The other set reflects the number of patients (in percent) in whom each parameter value increased after rTMS compared with before rTMS. If there were any increase post-rTMS compared with pre-rTMS value in any parameter, the value for that parameter was considered increased. If there were no change or any decrease in the post-rTMS compared with the pre-rTMS value in any parameter, the value for that parameter was considered decreased.

RESULTS

After rTMS, mean salivary CA VI activity and salivary zinc and copper concentrations increased significantly as did mean erythrocyte CA I, II activity and plasma copper concentrations (Table 1).

Significant increases in both CA I, II and CA VI activity were also measured [paired comparisons ($P < 0.01$, Student t test), sign test ($P < 0.05$, Student t test) (data not directly shown)]. These latter data are reflected in a summary of these results shown in Table 2 in which total changes before and after rTMS are shown with respect to the number of patients (in percent) who experienced either an increase or decrease in the parameters measured in Table 1 (see Methods section for details). Increased CA VI was measured in 90% of patients with changes varying from >0 to +153% (mean change, +17%) (Table 2); compared with chance, changes of this frequency and magnitude would occur <5 times in 1000 (χ^2), $P < 0.005$. Increased CA I, II was measured in 93% of patients