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Clozapine potentiation of GABA mediated cortical inhibition in treatment resistant schizophrenia

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ABSTRACT

Background: Cortical inhibition (CI) deficits have been demonstrated in schizophrenia using transcranial magnetic stimulation (TMS). These CI deficits may be related to decreased GABA activity which may be involved in schizophrenia pathophysiology. Previous cross-sectional studies have also demonstrated greater CI in patients treated with clozapine than other typical/atypical antipsychotics. However, it is not clear if these differences in CI are a result of treatment-resistant illness which necessitates clozapine or are related to clozapine treatment.
Methods: TMS measures of CI (i.e., cortical silent period (CSP) and short-interval cortical inhibition (SICI)) were measured over the motor cortex in 16 patients with schizophrenia before starting clozapine, then 6 weeks and 6 months after starting clozapine.
Results: CSP was significantly longer after 6 weeks of treatment with clozapine ($p = 0.014$). From 6 weeks to 6 months, there was no significant difference in CSP ($p > 0.05$). Short-interval cortical inhibition (SICI) was not significantly different at any time after treatment with clozapine ($p > 0.05$).
Conclusions: This prospective-longitudinal study demonstrates that treatment with clozapine is associated with an increase in $GABA_B$ mediated inhibitory neurotransmission. Potentiation of $GABA_B$ may be a novel neurotransmitter mechanism that is involved in the pathophysiology and treatment of schizophrenia.

1. Introduction

Schizophrenia is a debilitating illness that affects 0.3–0.7% of the population, and whose pathophysiology remains poorly understood (McGrath et al., 2008). Multiple converging lines of evidence in neuropathology, neurophysiology, and pharmacology suggest that persons with schizophrenia have deficits in cortical inhibition (CI). Neuropathological studies have shown a reduced number of gamma-aminobutyric acid (GABA) interneurons, which are cells that mediate CI (Del Río and DeFelipe, 1997). Neurophysiological studies also report CI deficits in persons with schizophrenia, as demonstrated by deficits in P50 auditory gating (Freedman et al., 2000). This P50 suppression is related to presynaptic $GABA_B$ receptors on excitatory neurons that input to pyramidal cells (Freedman et al., 2000). Pharmacologically, clozapine improves neurophysiological measures of CI (Daskalakis et al., 2008), which may be mediated by direct action of clozapine upon the $GABA_B$ receptor (Wu et al., 2011).

CI is thought to be mediated by two subtypes of GABA interneurons: $GABA_A$ and $GABA_B$. $GABA_A$ is the fast-acting ionotropic receptor (Macdonald and Olsen, 1994), while $GABA_B$ is the slow-acting metabotropic receptors (Bettler et al., 2004). The independent contribution to CI of each GABA subtype can be indexed using transcranial magnetic stimulation (TMS). $GABA_A$ inhibitory activity can be measured by short-interval cortical inhibition (SICI) (Kujirai et al., 1993), which consists of a subthreshold conditioning pulse preceding the suprathreshold pulse by 1–5 ms. In this scenario, motor evoked potential (MEP) response is inhibited by 50–90% (Kujirai et al., 1993). $GABA_B$ inhibitory activity can be measured by the cortical silent period (CSP) (Fuhr et al., 1991; Cantello et al., 1992). CSP measurement consists of motor cortex stimulation paired with voluntary electromyographic activity, resulting in cessation of muscle movement. The duration of muscle movement cessation (in milliseconds) is a measure of CI (Fuhr et al., 1991). Several lines of evidence suggest that CSP and SICI represent $GABA_B$- and $GABA_A$-mediated inhibition, respectively. First, the $GABA_B$ receptor-dependent IPSP peaks at 150–200 ms corresponding to the duration of the CSP while the $GABA_A$ receptor-dependent IPSP peaks at 20 ms corresponding to the duration of SICI (McCormick, 1989; Davies et al.,

Abbreviations: CI, cortical inhibition; CSP, cortical silent period; SICI, short-interval cortical inhibition; ICF, intracortical facilitation; GABA, gamma-aminobutyric acid; TMS, transcranial magnetic stimulation; MEP, motor evoked potential; EMG, electromyography; TS, testing stimulus; CS, conditioning stimulus; ISI, inter-stimulus interval.

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1990; Kang et al., 1994; Deisz, 1999). Secondly, the $GABA_B$ agonist baclofen prolongs the CSP (Siebner et al., 1998), while the $GABA_A$ agonist diazepam increases SICI (Ziemann et al., 1996). Finally, subthreshold conditioning stimuli are used to activate SICI, which indexes $GABA_A$ activity, while suprathreshold stimuli are used to activate CSP, which indexes $GABA_B$ activity. This is consistent with the finding that $GABA_A$-mediated IPSPs have lower activation thresholds than $GABA_B$-mediated IPSPs (Mody et al., 1994).

Two recent cross-sectional studies have reported that clozapine is associated with potentiation of $GABA_B$ inhibitory neurotransmission when indexed by TMS (Daskalakis et al., 2008; Liu et al., 2009). Specifically, patients on clozapine had significantly longer CSP than unmedicated patients (Daskalakis et al., 2008) and patients on other typical/atypical antipsychotics (Liu et al., 2009). However, both of these studies were cross-sectional in nature, therefore it is possible that the increased CI observed may be due to illness severity that necessitated clozapine treatment, rather than the effect of clozapine itself.

Previous work measuring $GABA_A$ mediated potentiation of clozapine has been less robust than that for CSP (Daskalakis et al., 2008; Liu et al., 2009). Initial work demonstrated that there was no significant difference in SICI between healthy volunteers, unmedicated patients with schizophrenia, and patients treated with clozapine (Daskalakis et al., 2008). There was only a slight non-significant increase in SICI (or smaller ratio) between unmedicated and clozapine groups. Subsequent work did not find significant differences between healthy subjects, unmedicated, typical/atypical antipsychotic, or clozapine groups (Liu et al., 2009). It was found however, that patients treated with clozapine had less SICI (or larger ratio) than healthy subjects and patients with schizophrenia receiving non-clozapine antipsychotics (Liu et al., 2009). This is consistent with the finding of clozapine's suppression of $GABA_A$ receptor mediated inhibitory neurotransmission (Michel and Trudeau, 2000), which may explain the increased risk of seizures for patients on clozapine (Devinsky et al., 1991).

The objective of this study was to measure TMS indices of CI (i.e., CSP and SICI) in patients with schizophrenia before and after clozapine treatment. Our primary hypothesis is that $GABA_B$ mediated CI (as measured by CSP) would be increased through clozapine and not simply associated with treatment resistance. We also explored whether any change in the CSP was associated with symptom improvement. Intracortical facilitation (ICF) was also investigated since ICF is thought to be related to NMDA mechanisms (Schwenkreis et al., 1999) – another potential neurochemical target of clozapine (Schwieler et al., 2008) – and since ICF may interact with the different inhibitory measures (Sanger et al., 2001; Daskalakis et al., 2002).

2. Materials and methods

2.1. Subjects

There were a total of 33 subjects in this study. Eighteen subjects were patients with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder, recruited at the Center for Addition and Mental Health (Toronto, Ontario, Canada). Inclusion criteria for this study were: (1) age between 18 and 65, (2) documented medication resistance, defined as documented treatment failure to adequate trials of at least 2 antipsychotic medications, including at least 1 atypical antipsychotic (Suzuki et al., 2012), and (3) willingness to switch to another antipsychotic medication, either clozapine or another non-clozapine antipsychotic as decided by their treating psychiatrist, with no input or interference from the study personnel. Exclusion criteria included a self-reported comorbid medical illness, history of drug or alcohol abuse/dependence, active suicidal ideation, or traumatic brain injury. The remaining 15 subjects were age and sex matched healthy subjects who were recruited as part of a separate study and were measured once at baseline.

2.2. Study design

This study was a prospective study. Clinical diagnoses of the subjects were confirmed using the Structured Clinical Interview for DSM-IV (SCID) (First et al., 2002). Patients were treated in an open-label fashion, and the medication was selected by the patient in consultation with their treating psychiatrist. In order to minimize influence on medication selection from the study protocol, the choice of medication was not revealed to study personnel until after medication selection was finalized. Prior to starting the new antipsychotic, for baseline measurements the patient was brought to the lab for neurophysiological testing and clinical assessment using the positive and negative syndrome scale (PANSS) (Kay et al., 1987). There was no washout period prior to starting the new antipsychotic and patients were immediately transitioned to either clozapine or a new antipsychotic. After starting the new antipsychotic, the patient was brought back to the lab at 6 weeks and 6 months to repeat the neurophysiological measurements and clinical assessments. Response to clozapine was defined as 20% reduction in PANSS score from baseline (Rosenheck et al., 1999). The protocol was approved by the Ethical Review Board of Centre for Addiction and Mental Health in accordance with the Declaration of Helsinki, and all subjects gave written informed consent.

2.3. Measurement of cortical inhibition

Surface electromyography (EMG) was recorded from the right abductor pollicis brevis (APB) muscle, and the signal was amplified (Intronix Technologies, Model 2024 F, Bolton, Canada), filtered (bandpass 2–2.5 kHz), and digitalized at 5 kHz (Micro 1401, Cambridge Electronics Design, Cambridge, United Kingdom).

TMS was applied to the left motor cortex, and motor threshold was determined according to a previously outlined protocol (Rossini et al., 1994). Briefly, focal TMS was administered with a 7-cm figure-of-eight coil using two Magstim 200 magnetic stimulators connected via a Bistim module (Magstim, Whitland, Dyfed, United Kingdom). The site of optimal motor response in the APB muscle was located by moving the coil to find the position that produced the largest MEP. The coil was placed on the spot and held tangential to the scalp with the handle pointing back and away from the midline at 45°. The current induced in the cortex was posterior–anterior, perpendicular to the line of the central sulcus.

The resting motor threshold (RMT), expressed as a percentage of maximum stimulator output, was determined as the lowest stimulation intensity that evoked peak-to-peak MEPs of 50 μV in at least 5 of 10 consecutive trials in the relaxed APB muscle. Measurement of the CSP was performed on an actively contracted APB muscle. The subjects were instructed to pinch a dynamometer with thumb and index finger to determine the magnitude of maximal APB contraction. Subjects were then asked to pinch with a force that kept the readings of the dynamometer at 20% of maximal contraction force. When TMS stimuli were delivered at 140% RMT at the site of optimal motor response, there was a pause of all muscle activities at the actively contracted APB. The absolute CSP duration was defined as the time from the MEP onset to the return of any voluntary muscular activity detected by EMG (Tergau et al., 1999). Ten TMS stimuli were delivered with an interstimulus interval (ISI) of 5 s, and mean CSP duration was calculated by averaging 10 CSP durations. The CSP duration was manually measured using Signal (v2.09, Cambridge Electronics Design, Cambridge, United Kingdom).

SICI and ICF were determined using paired-pulse TMS with recordings in the relaxed APB muscle according to published protocols (Kujirai et al., 1993). A subthreshold conditioning stimulus (CS) at 80% of RMT was followed by a suprathreshold test stimulus (TS) that was adjusted to produce mean peak-to-peak MEP amplitude of 1 mV. The inter-stimulus interval (ISI) determines whether the paired-pulse stimulus is assessing SICI or ICF. Specifically, an ISI of 2 or 4 ms measures SICI, while an ISI of 10, 15, or 20 ms measures ICF. The MEP amplitudes for