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The effect of noradrenergic attenuation by clonidine on inhibition in the stop signal task

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ABSTRACT

Understanding the neuropharmacology of inhibition is of importance to fuel optimal treatment for disorders such as Attention Deficit/Hyperactivity Disorder. The aim of the present study was to assess the effect of noradrenergic antagonism by clonidine on behavioral-performance and brain-activity indices of inhibition. A placebo-controlled, double-blind, randomized, crossover design was implemented. Male (N = 21) participants performed in a visual stop signal task while EEG was recorded under clonidine in one session and under placebo in another. We expected that 100 μg clonidine would have a negative effect on EEG indices of inhibition, the Stop N2 and Stop P3. Furthermore, we expected that clonidine would negatively affect the behavioral measure of inhibition, the stop signal reaction time (SSRT). Behavioral analyses were performed on data of 17 participants, EEG analyses on a subset (N = 13). Performance data suggested that clonidine negatively affected attention (response variability, omissions) without affecting inhibition as indexed by SSRT. Electrophysiological data show that clonidine reduced the Stop P3, but not the Stop N2, indicating a partial negative effect on inhibition. Results show that it is unlikely that the Stop P3 reduction was related to the effect of clonidine on lapses of attention and on peripheral cardiovascular functioning. In conclusion, the current dose of clonidine had a negative effect on attention and a partial effect on inhibitory control. This inhibitory effect was restricted to the dorsal region of the prefrontal cortex (presumably the superior frontal gyrus) as opposed to the ventral region of the prefrontal cortex (right inferior frontal gyrus).

1. Introduction

Mechanisms of inhibition are of obvious importance to everyday functioning. Abnormal functioning of mechanisms of inhibition is associated with disorders such as Attention Deficit/Hyperactivity Disorder (ADHD) (Kenemans et al., 2005; Pliszka et al., 2000). Methylphenidate is a standard pharmacological treatment for ADHD, and has been shown to positively affect inhibition and attention (Kenemans et al., 2005). Methylphenidate facilitates dopaminergic, but also noradrenergic neurotransmission by blocking the reuptake of these neurotransmitters (Zetterstrom et al., 1988). However, a significant number of patients suffer from side effects, hence, it is important to disentangle the relative contributions to both the clinical effect and side effects (Barkley, 1998). A better understanding of the role of the dopaminergic and noradrenergic system in attention and inhibitory control may fuel the development of more optimal pharmacological treatment.

Atomoxetine has also been used as a pharmacological treatment for reducing ADHD symptoms and is thought to facilitate noradrenergic neurotransmission by blocking noradrenaline reuptake (Del Campo et al., 2011). However, as a result of its effect on the noradrenaline transporter, atomoxetine does also increase prefrontal dopamine (Bymaster et al., 2002; Pliszka, 2005). Atomoxetine has been shown to positively affect inhibition, known to be a key component in ADHD (Chamberlain et al., 2007). More specifically, the effect of atomoxetine by enhancing noradrenergic neurotransmission on inhibition has been investigated using the stop signal task (SST) (Chamberlain et al., 2007; Chamberlain et al., 2006). In the SST, participants are required to respond to go stimuli which are infrequently followed by a rare stop signal after which a response to the go stimulus has to be withheld. This task yields the stop signal reaction time (SSRT), which is believed to be an index of inhibitory motor control. Results showed that atomoxetine decreased the SSRT, indicating facilitation of inhibition. However, in a more recent study, Graf et al. (2011) found that atomoxetine actually increased commission errors in a flanker go/no-go task. The number of such errors constitutes an alternative indication of failing inhibitory control. It must be noted though that it has convincingly been argued by Eagle et al. (2008) that the specific

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inhibitory component being taxed differs between the go/no-go and SST paradigm, which might explain differences between the results from Chamberlain et al. (2006) and Graf et al. (2011). Although speculation, in this vein, a reducing effect on one component of inhibition may result in a compensatory effect on the other. Alternatively, these seemingly contrasting findings may be explained by the inverted U relationship between NE levels and inhibitory performance. A relatively low dose of 60 mg apparently results in even more optimal NE levels both in healthy adults (Chamberlain et al., 2006) and adult ADHD patients (Chamberlain et al., 2007), but a relatively high dose of 80 mg (as in Graf et al., 2011) in healthy adults may yield supra-optimal NE levels with consequential reduced performance. In any case, it may be expected that decreased noradrenergic neurotransmission results in impaired inhibition. That is, NE antagonism would move the level of transmission away from the natural optimal level and result in monotonously decreasing performance. In the present study clonidine was used to attain a reduction in specifically noradrenergic transmission, and was compared to a placebo condition in which NE transmission was assumed to be at optimal levels. Clonidine is an agonist for both pre- and postsynaptic α2 receptors (Pliszka, 2005), and antagonizes spontaneous Locus Coeruleus–Noradrenaline (LC–NE) activity at low doses (Svensson et al., 1975).

The preferred measure for stopping performance, stop-signal reaction time or SSRT, cannot be measured directly and has to be inferred from stop rates and go-reaction time data. This estimation method is based on assumptions that are hard to verify (Band et al., 2003; Overtoom et al., 2002). In addition to behavioral measures, it is therefore advisable to use brain activity measures of inhibition. This also makes it possible to explain behavior in terms of brain activity. Indeed, in an attempt to localize the facilitation of stopping by noradrenergic enhancement by atomoxetine Chamberlain et al. (2009) showed, utilizing fMRI, that atomoxetine enhanced activity in the right inferior frontal gyrus (IFG). Importantly, the right inferior frontal gyrus (IFG) has been implicated as the neuroanatomical correlate of inhibition and related process of disengaging and reorienting of attention (Corbetta et al., 2008; Corbetta and Shulman, 2002). Research with patients with frontal-damage has indicated both right IFG and the superior frontal gyrus (SFG) to be implicated in intact inhibition (Aron et al., 2003; Floden and Stuss, 2006).

Electrophysiological indices of inhibition are readily recorded in the EEG. Most notable are the Stop N2 and Stop P3 Event Related Potentials (ERPs). Specifically, it has been shown that a stop signal synchronized brain potential at approximately 200 ms latency is significantly more negative on successful stop trials as opposed to failed stop trials (Schmajuk et al., 2006). This difference wave is termed the Stop N2. Furthermore, the right IFG has been implicated as the neurobiological correlate of the Stop N2 (Schmajuk et al., 2006). The Stop P3 is also modulated by stopping success, being larger for successful inhibitions as opposed to unsuccessful ones (Schmajuk et al., 2006). The Stop P3 has been interpreted as reflecting inhibition (Lansbergen et al., 2007) and is thought to originate from the superior frontal gyrus (SFG) (Kenemans and Kahkonen, 2011). In sum, using the SST in combination with EEG, a ventral inhibitory system as well as a more dorsal inhibitory system can be assessed as indexed by respectively the Stop N2 (right IFG) and Stop P3 (SFG).

In the current study we assessed whether noradrenergic attenuation by clonidine would negatively affect inhibition. A placebo controlled double-blind crossover design was used. Participants performed in the SST task while EEG was recorded. To our knowledge it is unknown whether clonidine is harmful for a fetus, which is why only men were included. With respect to brain activity measures of inhibition, it was expected that clonidine would attenuate the Stop N2 and Stop P3. With respect to behavioral measures, it was expected that clonidine would increase the SSRT. In addition, performance (omission errors and reaction-time fluctuation), as well as subjective measures of alertness were recorded to monitor the potentially sedative effects of clonidine.

2. Methods

2.1. Participants and drug treatment

It was required that participants pass a medical screening consisting of an interview and a cardiovascular assessment. Specifically, participants with a blood pressure under 100 mm Hg systolic and/or 70 mm Hg diastolic and/or heart rate below 60 or above 100 bpm were excluded. Participants had normal or corrected to normal vision. A total of 21 male healthy subjects (age, M: 22 SD: 3) were included in the study. The study was approved by the local medical ethics committee of the University Medical Center Utrecht, and conducted in accordance with the declaration of Helsinki. Clonidine was used to attenuate noradrenergic signaling. According to the Summary of Product Characteristics (Centrafarm Services B.V., Etten-Leur, The Netherlands) for clonidine, maximum plasma levels are reached after 1 to 3 h. Clonidine is relatively long acting and, according to the SPC, has a half-life of approximately 9 h. Initially a dosage of 200 μg of clonidine was used. However, the first two participants receiving clonidine experienced significant side effects and did not complete the experiment. More specifically, one participant became unwell, possibly due to the significant drop in systolic and diastolic blood pressure and the other participant fainted (possibly a vasovagal collapse). Because of medical ethical concerns, the experiment was continued with 100 μg clonidine. From this sample, two participants were excluded. One participant had to vomit after capsule intake, the other participant presented with cardiac arrhythmia and slow pulse after the pretest (but before treatment). In total, 17 subjects completed the experiment.

2.2. Stop signal task (SST)

The SST was modeled after the SST reported in Schmajuk et al. (2006). The primary task consisted of a dual choice task in which go-stimuli (the letters "X" and "O"; visual angles: (h) 1.4° × (w) 1.4° and 1.4° × 1.3° respectively) were presented randomly and sequentially. The letters were presented for 150 ms, centrally and slightly above a continuously present fixation cross. Participants had to discriminate between the go stimuli by pressing the left or right button on a response board with the left or right index finger. The trial-to-trial interval was varied between 1.5 and 1.8 s. The experiment consisted of a pretest (before capsule administration) and posttest (after capsule administration). The pretest consisted of 4 blocks, one practice block, consisting of 126 go trials, and three stop signal blocks consisting of 128 trials. In 25% of trials in the stop signal blocks, a go stimulus was followed by a stop stimulus consisting of a "$" sign (visual angle: 1.7° × 0.8°), presented at the same location as the go stimuli. The first block was used as a practice block and to establish a baseline average reaction time. If participants slowed more than 1.5 times this baseline reaction time in subsequent stop-signal blocks, participants were instructed to speed up, but only if more than 40% inhibitions were made. After the practice block, a base stop signal block was presented in which the go-stop Stimulus Onset Asynchrony (SOA) was fixed at 250 ms. Subsequently, two experimental stop signal blocks followed. After each stop-signal block, the go-stop SOA was dynamically adjusted based on the stop rate in the previous block to ensure an approximate 50% stop rate (De Jong et al., 1995). If the stop rate was below 40% (even after dynamic SOA adjustment), participants were instructed to respond slightly slower to the go-stimuli. The posttest was similar to the pretest but consisted of 9 blocks. The posttest started with a practice block followed by the base stop signal block and three experimental stop signal blocks. After the stop signal blocks, the response–stimulus assignment was switched and four equivalent stop signal blocks (base block plus three experimental blocks) were presented again. In both the pretest and posttest, the stop signal was jittered over 99 ms below and above the set SOA in the experimental stop signal blocks to allow