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Pindolol does not augment central serotonin function increases to citalopram in humans: An auditory evoked potential investigation

Rebecca Segrave [a], Rodney J. Croft [a,c], Susan Illic [a], K. Luan Phan [b,c], Pradeep J. Nathan [c,*]

[a] Brain Sciences Institute, Swinburne University of Technology, Melbourne, Australia
[b] Clinical Neuroscience and Psychopharmacology Research Unit, Department of Psychiatry, University of Chicago, Chicago, IL, USA
[c] Behavioural Neuroscience Laboratory, Department of Physiology and Monash Centre for Brain and Behaviour, P.O. Box 13F, Monash University, VIC, 3800, Australia

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Abstract

Animal studies have demonstrated that the co-administration of pindolol and selective serotonin reuptake inhibitors (SSRIs) potentiate serotonergic functioning to a greater degree than SSRIs alone. However, clinical trials of pindolol augmentation in patients with major depressive disorder have reported contradictory findings, and the central effects of this treatment regime on serotonin functioning in humans are unknown. The current double-blind placebo controlled repeated measures investigation used the loudness dependence auditory evoked potential (LDAEP) to assess central serotonin functioning in healthy participants across three acute treatment conditions: placebo, citalopram (20 mg), and pindolol (10 mg)+citalopram (20 mg). The current paper focuses on the effects of pindolol augmentation of citalopram as compared to the administration of citalopram alone. Enhancement of serotonin function with citalopram in comparison to placebo decreased the slope of the LDAEP (i.e. weaker LDAEP). However, there were no significant differences between the changes in the LDAEP induced by co-administration of pindolol and citalopram compared to citalopram. The present results indicate that, in healthy controls, pindolol augmentation of SSRIs does not potentiate central serotonin function to a greater degree than the administration of an SSRI alone. The findings may provide further support for why pindolol may not be an effective therapeutic strategy to augment serotonin function and antidepressant response.

Keywords: Serotonin; Selective serotonin reuptake inhibitor; Citalopram; Pindolol; Loudness dependence auditory evoked potentials; 5-HT_{1A} receptor

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are now the most commonly prescribed class of antidepressants for the treatment of major depressive disorder (Sclar et al., 1998; Lawrenson et al., 2000; Hemels et al., 2002). The major limitation of antidepressant drugs including SSRIs is that while they block serotonin reuptake almost immediately following administration, patients frequently do not begin to experience a therapeutic response until 2–4 weeks post treatment initiation (Sugrue, 1983; Leonard, 1984).

The prevailing theory concerning the latency of clinical response following SSRI administration focuses on adaptational changes in pre-synaptic somatodendritic 5-HT_{1A} receptors (autoreceptors) in the dorsal raphae nucleus. The increase in extracellular serotonin availability induced by acute SSRI administration stimulates pre-synaptic 5-HT_{1A} receptors, initiating a negative feedback loop whereby the firing of serotonin neurons is inhibited, resulting in a reduction of serotonin in the synaptic cleft and at cortical projection areas. Experimental evidence suggests that sustained stimulation of pre-synaptic 5-HT_{1A} receptors results in their functional desensitisation (for review, see Hensler, 2003), and the consequential disinhibition of neuronal firing leads to a rise in synaptic, and therefore cortical, serotonergic concentration (Artigas, 1993; Blier and de Montigny, 1994). The 2–4 weeks that are generally thought to be necessary for desensitisation to occur correlate well with the onset of antidepressant action (for review see Blier and de Montigny, 1994; Artigas et al., 1996).

* Corresponding author. Tel.: +61 9905 2553; fax: +61 9905 2547.
E-mail address: Pradeep.Nathan@med.monash.edu.au (P.J. Nathan).

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2. Pindolol augmentation

Pindolol is a mixed β-adrenergic antagonist, with additional antagonistic properties at the 5-HT_{1A} receptor (Clark et al., 1982; Clifford et al., 1998; Newman-Tancredi et al., 1998). It has been suggested that the co-administration of pindolol with SSRIs may represent a means to both accelerate and augment the effect of SSRIs via blockade of pre-synaptic 5-HT_{1A} receptors, thus bypassing the aforementioned negative feedback loop (Artigas, 1993; Hjorth and Sharp, 1993; Blier and de Montigny, 1994), and hastening antidepressant response. The results of a number of studies in both animals and humans provide support for this theory. Microdialysis studies in rats have shown that the combined administration of an SSRI with selective 5-HT_{1A} receptor antagonists increase extracellular serotonin levels in projection areas such as the frontal cortex and dorsal striatum, to a greater degree than administration of an SSRI alone (Dreshfield et al., 1996; Hjorth, 1996; Romero et al., 1996; Dawson and Nguyen, 1998). In patients with major depressive disorder (MDD), a number of open label studies have reported a significant reduction in the latency of therapeutic response following the co-administration of pindolol with SSRIs, in some cases to less than a week. Augmentation of antidepressant response has also been reported in patients with MDD who are treatment resistant (Blier and Bergeron, 1995; Bakish et al., 1997).

A number of double-blind placebo controlled studies in patients with MDD have reported a reduction in the time to antidepressant response to 2 weeks or less following co-administration of pindolol and SSRIs (Bordet et al., 1998; Smeraldi et al., 1998; Zanardi et al., 1998, 2001; Perez et al., 2001), but not all trials have found a reduction in the therapeutic lag (Moreno et al., 1997; Tome et al., 1997; Berman et al., 1999; Perez et al., 1999; Perry et al., 2004). Augmentation of antidepressant response with concurrent pindolol and SSRI administration has also been reported (Perez et al., 2001; Zanardi et al., 2001), but again incongruent findings have been described (Moreno et al., 1997; Tome et al., 1997; Bordet et al., 1998; Perry et al., 2004). The pattern emerging from these studies is that pindolol accelerates antidepressant response to SSRIs under certain circumstances (for review see Segrave and Nathan, 2005), but factors facilitating improved antidepressant response and the effects of pindolol augmentation on central serotonin function in the human brain remain unclear.

One potential explanation for the variability in the results of clinical trials to date relates to the 2.5 mg t.i.d. (7.5 mg/day) dose of pindolol that has been administered in all but one (Bordet et al., 1998) of these investigations. A recent study using positron emission tomography (PET) reported typically low, and highly variable (mean = −1% ± 24%), occupancy of pre-synaptic 5-HT_{1A} receptors in the dorsal raphae nucleus following the administration of 2.5 mg t.i.d. pindolol. Further, it was reported that at 2.5 mg t.i.d. pindolol did not exhibit preferential occupancy of 5-HT_{1A} pre-synaptic over postsynaptic receptors (Rabiner et al., 2001; Rabiner et al., 2004), which is of critical importance in pindolol augmentation. A dose of pindolol that preferentially blocks pre-synaptic 5-HT_{1A} receptors will facilitate SSRI induced increase in serotonin in the synaptic cleft, and transmission onto cortical projection areas, without blocking post-synaptic sites. Blockade of postsynaptic 5-HT_{1A} receptors is undesirable as postsynaptically 5-HT_{1A} receptors mediate the effects of serotonin transmission from nerve terminals, and their blockade can be expected to be associated with a reduction in antidepressant response (Blier et al., 1997). It has been suggested that the administration of pindolol in higher doses (i.e. greater than 7.5 mg/day) may more reliably augment antidepressant response to SSRIs (Berman et al., 1997, 1999; Perez et al., 1999). Supporting this, the administration of pindolol 10 mg s.o.d. (but not 5 mg or 20 mg) in healthy participants has been found to result in significant pre-synaptic 5-HT_{1A} occupancy, and to occupy significantly more pre than postsynaptic receptors (Rabiner et al., 2004).

While the aforementioned PET investigations have examined the effects of pindolol and SSRI co-administration on 5-HT_{1A} receptor binding, no studies to date have directly investigated the effects of pindolol augmentation of SSRIs on central serotonin functioning in humans. The majority of human research in this area continues to rely on behavioral measures to assess the effects of pindolol augmentation in clinical populations. A better understanding of the central serotonergic effects of this treatment regime is necessary if it is to be reliably employed in a clinical setting. Because peripheral indices such as serotonin serum levels and metabolites, do not fully reflect central serotonin functioning (Murphy, 1990), it is preferable to use central measures when investigating modulation of central serotonin functioning. One of the major limitations facing investigations examining central serotonin functioning in the human brain is the lack of reliable non-invasive measures of serotonin function.

3. The loudness dependence auditory evoked potential

The loudness dependence auditory evoked potential (LDAEP) has been suggested as a possible measure of central serotonin function (for review, see Hegerl et al., 2001). The LDAEP is a measure of auditory cortex neuronal activity, reflecting an increase or decrease in the slope relating the amplitude change of the auditory evoked potentials to different stimulus intensities. High serotonergic neurotransmission, resulting from a high firing rate of serotonergic neurons in the dorsal raphae nucleus, is thought to result in a weak LDAEP, a small increase in the evoked cortical response with increasing loudness of the stimuli. A low serotonergic neurotransmission on the other hand is said to be related to a strong LDAEP (Hegerl et al., 2001).

Taken together, the results of a large number of studies provide strong support for a relationship between the LDAEP and serotonin functioning. A stronger LDAEP (i.e. a pronounced increase in the slope of the N1/P2 waveform with increasing tone loudness) has been associated with low levels of the serotonin metabolite 5-hydroxy-indole-acetic-acid (5-HIAA) (von Knorring and Perris, 1981), and observed following local application of the 5-HT_{1A} agonist 8-OH-DPAT in the dorsal raphae nucleus (Juckel et al., 1999), and

the intravenous administration of the 5-HT_2/5-HT_{1C} antagonist ketanserin (Juckel et al., 1997). Similarly a stronger LDAEP is found in conditions associated with low serotonin, including those with the ss genotype of the serotonin transporter gene (5-HTTLPR) (Gallinat et al., 2003), patients with depression (Buchsbaum et al., 1971), and MDMA users (Tuchtenhagen et al., 2000; Croft et al., 2001). A weaker LDAEP (a flatter N1/P2 slope with increasing tone loudness), has been found following administration of the 5-HT_{1A} antagonist spiperone in the dorsal raphae nucleus (Juckel et al., 1999), stimulation of postsynaptic 5-HT_{1A} receptors with 8-OH-DPAT (Juckel et al., 1997), after augmentation of serotonin with chronic sertraline administration (Simmons et al., 2000), and in patients with generalized anxiety disorder (Senkowski et al., 2003). A positive correlation has also been reported between the LDAEP and severity of serotonin syndrome symptoms (Hegerl et al., 1998). Of clinical interest is that in patients with depressive disorders, a positive correlation has been reported between LDAEP and favorable response to SSRIs and lithium (Hegerl et al., 1992; Paige et al., 1994; Gallinat et al., 2000; Juckel et al., 2004).

The current study aimed to use the LDAEP to examine the effects of pindolol augmentation of citalopram on central serotonin functioning. It was predicted that in healthy participants, a weaker LDAEP (indicative of increased serotonin functioning) would be observed following acute SSRI administration as compared to placebo; and that a further decrease in the LDAEP would be observed following co-administration of pindolol 10 mg s.o.d. and citalopram (indicative of a further increase in serotonergic functioning).

4. Methods

4.1. Participants

Twelve healthy participants (7 males; aged 21–31 years, mean=23±3 years) were recruited via advertisements placed around Swinburne University in Melbourne. One participant withdrew following the second testing session and was consequently omitted from analysis. All participants had normal or normal-to-corrected vision and no hearing impairments. They were non-smokers, drug free (including the oral contraceptive pill) and free of any physical illness, psychopathology and history of mental illness or substance abuse, as determined by telephone screening using the Prime-MD (DSM-IVTM evaluation guide, Pfizer Inc.) and a subsequent semi-structured medical examination and interview conducted by a medical physician. All subjects gave written informed consent to participate in the study, which was approved by the Swinburne Human Research Ethics Committee.

4.2. Study design and procedure

The study employed a double blind, placebo controlled, double dummy, repeated measures design. Each participant was tested under three separate acute treatment conditions; placebo, citalopram (20 mg s.o.d.), and combined pindolol (10 mg s.o.d.) and citalopram (20 mg s.o.d.). The timing of drug administration was structured so that during the pindolol and citalopram condition, pindolol was administered 30 minutes before citalopram, to enable blockade of pre-synaptic 5-HT_{1A} receptors prior to citalopram administration. The timing of drug administration was chosen to coincide with peak plasma levels for both pindolol (t_{max}=1–3 h; Rabiner et al., 2001; Martinez et al. 2001) and citalopram (t_{max}=2–4 h; Hyttel, 1994). Citalopram was chosen as it is the most selective SSRI (Hyttel, 1994). A 20 mg dose was used as it is the minimum recommended starting dose to reduce the chance of side effects. This dose has previously been shown to induce central electrophysiological effects (Kemp et al., 2004). Pindolol 10 mg was administered as, in healthy subjects, it has been demonstrated in PET studies that this dose achieves a significant occupancy of 5-HT_{1A} receptors while binding preferentially to pre-synaptic over postsynaptic receptors (Rabiner et al., 2000, 2004). The order of treatment administration was randomised according to a Latin Square design.

Each of the three testing sessions was separated by a minimum 1-week wash out period. To avoid the acute effects of alcohol or caffeine from confounding test results all participants were required to abstain from alcohol and caffinated products 24 h prior to testing. To control for the possible influence of phase-dependant hormonal fluctuations in female participants they were tested during the follicular phase of their menstrual cycle (days 1–13). The timing of testing sessions was constant across all participants for all sessions.

Participants arrived at approximately 9:00 am on testing days, at which time they completed a modified version of the Visual Analogue Mood Scale (VAMS; Bond and Lader, 1974; which was administered to determine whether any effects on the LDAEP were confounded by mood). Five subscales of the VAMS were employed: happy–sad, content–discontent, sociable–withdrawn, tranquil–troubled, amicable–antagonistic, and were chosen as they represent commonly observed serotonergic mood changes (Bhatti et al., 1998; Barton et al., 2003; Hughes et al., 2004). Immediately afterwards participants were given their first capsule, containing either placebo or pindolol 10 mg. Thirty minutes after the administration of the first capsule a second capsule containing either placebo or citalopram 20 mg was administered. Two hours after administration of the second capsule electroencephalography (EEG) recording commenced. Finally, after completion of the EEG recording, the modified VAMS was re-administered.

4.3. Data acquisition

Participants were seated upright in a comfortable chair with their eyes open, in a sound attenuated and electrically shielded room. They were instructed to relax throughout the recording and to avoid muscle movements throughout the stimulus presentation sequence. EEG was recorded with tin electrodes from 66 scalp sites according to the international 10/20 system, and eye movement activity from an electrode below the left eye, using CZ as a reference and AFZ as ground. In addition, electromyography (EMG) was recorded from two electrodes beneath the right eye field. Fifty stimulus tones (1000 Hz, 50 ms duration including 10 ms rise and 10 ms fall, SOA randomised

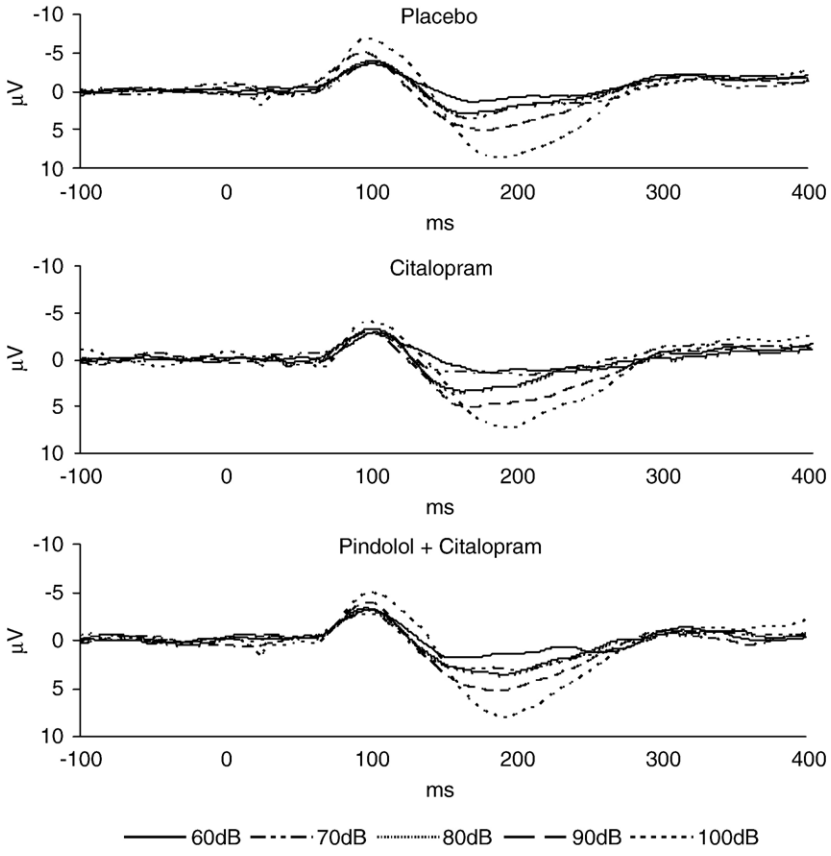

Fig. 1. Grand mean auditory evoked potentials at Cz for each intensity of auditory stimulus, following treatment with placebo, citalopram and pindolol+citalopram.

between 1600 ms and 2100 ms) at each of five intensities (60, 70, 80, 90, 100 dB generated by a Stim II sound generator) were presented binaurally in a pseudorandomised form through single use foam EAR inserts. During the presentation of auditory stimuli participants were presented with a series of faces and asked to respond via a button press if the face had a nose (they were asked to ignore faces without a nose). The purpose of this visual task was to distract attention away from the auditory stimuli, as attention has been shown to modulate the intensity dependence of evoked potentials in humans (Baribeau and Laurent, 1987; Carrillo-de-la-Pena, 1999). Data was collected with a sampling rate of 1000 Hz, and a bandpass filter of 0.15–200 Hz.

4.4. Data analysis

4.4.1. EEG analysis

EEG data collection and auditory stimulus presentation were conducted using Neuroscan 4.3 acquisition hardware and Neuroscan Stim 4.3 software. EEGs were recorded using tin electrodes and ECI Electro-gel, and all impedances were below 5 kΩ. EEG data were re-referenced to linked mastoids, lowpass filtered at 30 Hz (12dB/oct) and the Croft and Barry (2000) method employed to correct for ocular artifact. Data was then visually inspected and rejected where artifactual, and epoched-100–600 ms post auditory stimulus (discarding the first 5 stimuli to reduce novelty effects). Averages were then created for each of the five intensities separately, with a mean of 47.81 ± 2.23 sweeps per intensity. N1-peaks (80–120 ms) and P2-peaks (150–230 ms) were determined automatically at the CZ electrode. N1/P2 amplitude was calculated as the difference in amplitude between N1 and P2. The slope of the N1/P2 was calculated as the linear regression slope, with stimulus intensity as the independent variable and N1/P2 amplitude as the dependant variable. The investigator analysing the AEP data was blind to the coding of the treatment conditions.

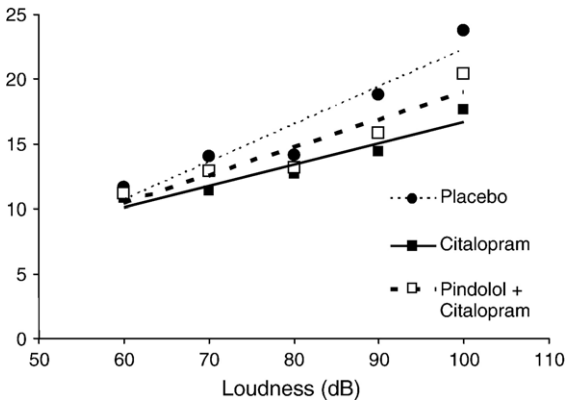

Fig. 2. Mean N1/P2 amplitude is plotted against stimulus intensity, for the placebo, citalopram, and the pindolol+citalopram conditions separately. Least-squares regression lines are also shown, where it can be seen that the slope of the citalopram condition shows a less steep slope than the other two conditions.

Table 1
Mean, standard deviation, median and range for VAMS difference scores (pre treatment − post treatment) by treatment condition

VAMS	P			C			PC		
	Mean (SD)	Median	Range	Mean (SD)	Median	Range	Mean (SD)	Median	Range
Happy–sad	0.29 (1.01)	0.15	−1.20–2.10	0.10 (1.37)	0.05	−2.80–2.10	0.41 (1.02)	0.50	−1.80–2.40
Content–discontent	0.03 (0.92)	0.35	−1.40–1.50	0.37 (1.81)	0.85	−3.30–2.40	0.27 (1.11)	0.50	−2.00–2.40
Sociable–withdrawn	0.31 (0.88)	0.25	−0.80–2.40	0.31 (0.88)	0.15	−2.20–2.30	0.41 (1.02)	0.50	−1.80–2.40
Tranquil–troubled	0.04 (0.75)	0.05	−1.30–1.00	0.49 (2.04)	0.6	−3.60–2.90	0.36 (0.88)	−0.10	−1.40–1.40
Amicable–antagonistic	0.25 (0.79)	0.30	−0.90–1.80	−0.37 (1.15)	−0.45	−3.60–2.90	−0.16 (0.89)	−0.40	−1.10–2.10

P = placebo, C = citalopram, PC = pindolol + citalopram; $p > 0.01$ (Bonferroni corrected α) for all VAMS difference scores between treatment conditions.

4.4.2. Statistical analysis

All statistical analysis was conducted using SPSS Version 12.0. Because data was not normally distributed the slope of the N1/P2 was normalized for all treatment conditions using a square root transformation (Tabachnick and Fidell, 2001) t_variable = [square root (old variable + 0.20)]. Repeated measures ANOVA was employed predicting a significant reduction in the slope of the N1/P2 in the combined pindolol and citalopram condition relative to the citalopram condition. Repeated measures ANOVA was used to ensure that there were no significant pre-existing differences in participant's mood prior to treatment administration, with mood as the dependant variable and treatment condition as the independent variable. The Greenhouse-Geisser correction for sphericity was used where appropriate. Using Wilcoxon Signed Rank test's (one-tailed) with a bonferroni corrected α of 0.01, the five scales were then analysed separately using VAMS difference scores (i.e. pre treatment score − post treatment score) across the placebo and the citalopram conditions, and the citalopram and pindolol + citalopram conditions. Finally, bivariate correlations were conducted to examine the relationship between each of the five VAMS mood scales and the treatment conditions.

5. Results

As was described in Nathan et al. (2006), a significant decrease in the slope of the N1/P2 with increasing tone loudness was observed during the citalopram treatment condition, relative to the placebo condition ($F(1, 10) = 9.34$, $p = 0.006$). In terms of the effect of pindolol on the citalopram condition, no significant difference in the loudness dependence of the N1/P2 slope was observed between the citalopram and the combined pindolol and citalopram treatment conditions ($F(1, 10) = 1.068$, $p = 0.163$). Fig. 1 illustrates the mean auditory evoked potentials at Cz for each level of intensity of auditory stimulus, following each treatment condition. Fig. 2 shows the mean N1/P2 slope following each treatment condition.

No significant difference was observed in any of the VAMS scales prior to treatment administration: happy–sad ($F(2, 22) = 0.12$, $p = 0.89$), content–discontent ($F(1.63, 17.96) = 0.33$, $p = 0.68$), sociable–withdrawn ($F(2, 22) = 0.25$, $p = 0.78$), tranquil–troubled ($F(2, 22) = 0.93$, $p = 0.41$), amicable–antagonistic ($F(1.61, 30.15) = 0.63$, $p = 0.51$). No change was observed in VAMS difference scores between the placebo and the citalopram treatment conditions for any of the scales: happy–sad ($z = -0.27$, $p = 0.40$), content–discontent, ($z = -0.89$, $p = 0.19$), sociable–withdrawn ($z = -1.07$, $p = 0.14$), tranquil–troubled ($z = -0.80$, $p = 0.21$), amicable–antagonistic ($z = -1.51$, $p = 0.07$). Similarly, no change was observed in VAMS difference scores between the citalopram and the combined pindolol and citalopram treatment conditions for any of the scales: happy–sad ($z = -1.61$, $p = 0.05$), content–discontent, ($z = -0.63$, $p = 0.27$), sociable–withdrawn ($z = -1.23$, $p = 0.10$), tranquil–troubled ($z = -0.24$, $p = 0.41$), amicable–antagonistic ($z = -0.83$, $p = 0.21$). Finally, no significant correlations were observed between any of the five VAMS mood scales and the treatment conditions. Table 1 lists the mean, standard deviation, median and range for VAMS difference scores (pre treatment − post treatment) by treatment condition.

6. Discussion

Acute enhancement of serotonin with citalopram resulted in a reduction of loudness dependence of the N1/P2 (weaker LDAEP), consistent with previous studies (Hegerl et al., 1998; Juckel et al., 1999; Simmons et al., 2000; Senkowski et al., 2003) showing an inverse relationship between serotonin function and the LDAEP (findings published in a previous paper; Nathan et al., 2006). This finding provides further evidence for a relationship between serotonin function and the LDAEP. The focus of this paper was to examine if the addition of pindolol to the SSRI citalopram enhanced central serotonin function to a greater extent than citalopram alone using the LDAEP. No significant change in the loudness dependence of the N1/P2 was observed following the combined administration of pindolol and citalopram (in comparison to citalopram alone), suggesting that this augmentation strategy did not enhance central serotonin function to a greater extent. No significant difference was observed in any of the VAMS scales prior to treatment administration, suggesting that the results were not influenced by pre-existing variations in participant mood state, which may have been expected to influence pre-treatment serotonergic activity (Bhatti et al., 1998; Barton et al., 2003; Hughes et al., 2004). No change was observed in participant's subjective ratings of mood following the administration of either placebo, citalopram, or combined pindolol and citalopram administration. Additionally, no significant correlations were observed between any of the measured mood states and treatment condition, further indicating the current findings were not related to changes in subjective mood state.

Interestingly, while the results of a number of animal microdialysis studies (Dreshfield et al., 1996; Hjorth, 1996; Romero et al., 1996), clinical open label investigations (Artigas et al., 1994; Blier and Bergeron, 1995; Bakish et al., 1997) and controlled clinical trials in patients with MDD (Tome et al., 1997; Zanardi et al., 1997, 1998, 2001; Bordet et al., 1998; Smeraldi et al., 1998; Perez et al., 2001) have provided support for pindolol augmentation of SSRIs, the current investigation found that the co-administration of pindolol and citalopram did not augment central serotonin functioning (indexed by the LDAEP) compared to citalopram alone. One explanation for this finding may relate to the magnitude of pre-synaptic 5-HT_{1A} receptor occupancy achieved by pindolol. The augmentation of SSRIs by pindolol is reliant on preferential binding at 5-HT_{1A} receptors (i.e. preferential occupancy of pre-synaptic over postsynaptic 5-HT_{1A} receptors). Accordingly, the present investigation used a 10 mg s.o.d. of pindolol that, in healthy participants, has been found to occupy a significantly greater proportion (mean = 35% ± 15%) of pre-synaptic 5-HT_{1A} receptors in the dorsal raphae nucleus than postsynaptic 5-HT_{1A} receptors (mean = 12.4% ± 13%) (Rabiner et al., 2000, 2004). On the basis of studies in rats (Romero et al., 1996; Corradetti et al., 1998) it has been estimated that between 50% and 75% occupancy of the pre-synaptic 5-HT_{1A} population may be required for reliable augmentation of SSRIs by pindolol (Martinez et al., 2001; Rabiner et al., 2001). These estimates are both substantially greater than the 35% reportedly achieved by the 10 mg s.o.d. dose used in the current investigation. Accordingly, insufficient pre-synaptic 5-HT_{1A} occupancy by pindolol may explain the lack of potentiation of central serotonin functioning following the combined administration of pindolol and citalopram.

If augmentation of SSRIs by pindolol is dependant on an increased proportion of pre-synaptic 5-HT_{1A} receptor occupancy, this would suggest that higher doses of pindolol need to be administered in order to ensure a greater proportion of autoreceptor blockade. PET investigations examining pindolol binding at 5-HT_{1A} receptors have demonstrated a dose-dependant increase in pre-synaptic occupancy (Rabiner et al., 2000, 2004; Martinez et al., 2001), and it has been estimated that a 15–20 mg/day dose would be required to block 50% of pre-synaptic 5-HT_{1A} receptors in the dorsal raphae nucleus (Martinez et al., 2001). However, while the administration of increased doses of pindolol is expected to result in increased occupancy of pre-synaptic 5-HT_{1A} receptors, the majority of PET investigations examining receptor binding following pindolol administration have found a positive correlation between dose and reduction in preferential occupancy. Following the administration of a 20 mg s.o.d. of pindolol in healthy participants, Rabiner et al. (2000) reported significant occupancy of pre-synaptic 5-HT_{1A} receptors (mean = 36% ± 3%), but found that at this dose it resulted in greater postsynaptic (mean = 46% ± 10%) than pre-synaptic occupancy. Further, in patients with MDD, preferential occupancy of 5-HT_{1A} receptors was not observed following the chronic administration of a pindolol 5 mg t.i.d. (15 mg/day) dosing regime (19% ± 18% occupancy in the dorsal raphae nucleus vs. 26% ± 13% postsynaptic occupancy) (Rabiner et al., 2004). Finally, administration of higher doses of pindolol is likely to result in cardiovascular side effects, such as lowered blood pressure and reduced heart rate, resulting from pindolol's non-selective β-adrenergic antagonistic properties and as such may preclude their administration in a clinical setting.

In order to avoid the aforementioned side effects clinical investigations of pindolol augmentation of SSRIs have typically employed a pindolol 2.5 mg t.i.d. dosing regime. However, as previously discussed, evidence from PET studies indicates that at this dose pindolol produces only modest and highly variable occupancy of pre-synaptic 5-HT_{1A} receptors, and does not preferentially occupy these over post-synaptic receptors (Rabiner et al., 2001). These PET findings suggest that the 2.5 mg t.i.d. dose of pindolol used in clinical trials of pindolol augmentation may be too low to consistently augment serotonin functioning, and thus the therapeutic effects of SSRIs. While the current investigation used healthy participants, the results are consistent with this idea. Thus it is possible that the dose of pindolol employed in clinical trials is contributing to the heterogeneity in the reported findings. Of interest is a recent study using a new controlled release formulation of pindolol that reported preferential occupancy of pre-synaptic 5-HT_{1A} receptors following a stepwise administration of controlled release pindolol to 30 mg/day over a period of 9 days (Martinez et al., 2001). As higher doses of a controlled release formulation would be associated with reduced side effects in comparison with repeated administration of the common immediate release pindolol, the administration of controlled release pindolol may represent a more tolerable and efficacious means of augmenting response to SSRIs, and warrants further investigation.

An additional explanation for the failure of pindolol to potentiate central serotonergic functioning in the current investigation may be related to its pharmacological properties (i.e. intrinsic activity). In addition to its antagonistic properties at 5-HT_{1A} receptors, pindolol has also been found to have partial agonistic effects. For example, electrophysiological studies in rats have suggested that pindolol may display dose-related agonist effects at the pre-synaptic 5-HT_{1A} receptors leading to a reduction in serotonin neuronal activity (Clifford et al., 1998; Gartside et al., 1999; Arborelius et al., 2000). As the current study employed a higher dose of pindolol than used in clinical investigations it is possible that pindolol was acting as an agonist rather than an antagonist at pre-synaptic 5-HT_{1A} receptors, and thus inhibiting rather than enhancing serotonergic firing rate. While the current study was not able to determine whether pindolol was acting as an antagonist or partial agonist at pre-synaptic 5-HT_{1A} receptors, it cannot be discounted that pindolol was acting agonistically, thus inhibiting rather than facilitating the activation of serotonin neurons.

The current study has a number of limitations. Firstly, the present investigation did not directly assess the magnitude of 5-HT_{1A} receptor binding following pindolol administration. Accordingly, we cannot be certain that the pre-synaptic or

preferential occupancy of 5-HT_{1A} receptors achieved in the current sample is equivalent to that reportedly achieved by PET investigations. Two previous PET studies (Rabiner et al., 2000, 2004), both examining healthy participants have reported preferential and equivalent binding at pre-synaptic 5-HT_{1A} receptors following a 10 mg s.o.d. pindolol and while it is possible that comparable occupancy was achieved in the current study, it is equally possible that given the large variation in receptor binding reported previously in studies with small sample sizes (Rabiner et al., 2000, 2004), that in our small sample, similar variation may exist and thus we may have not achieved desirable occupancies.

Additionally, the current study was conducted using a small sample of healthy participants, rather than patients with MDD. Accordingly, caution must be taken when making inferences from the current findings in normal participants in regard to the results of clinical trials of pindolol augmentation. Replication of the current study in larger and clinical populations may help to resolve this issue and such studies are currently underway in our laboratory. Finally, the present investigation computed the LDAEP from a single centrally located electrode (Cz) rather than conducting a more complex dipole source analysis (DSA). DSA analysis allows the separation of the auditory evoked N1/P2 component into a subcomponent mainly generated by the primary auditory cortex, and a subcomponent mainly generated by the secondary auditory cortex. In comparison with the secondary auditory cortex, the LDAEP of the primary auditory cortex is thought to be a more sensitive measure of central serotonin functioning, and as DSA allows this component to be examined separately, it may be a more sensitive measure than the one employed in the present study. However, the advantage of DSA in comparison to measurement from Cz has not been directly demonstrated, and similar results have previously been reported using the two methods. For example, similar enhancements of the LDAEP were found in long-term ecstasy users using vertex (Croft et al., 2001), and dipole analyses (Tuchtenhagen et al., 2000).

In conclusion, the current study found that in comparison with acute citalopram administration, the co-administration of pindolol and citalopram was not associated with a change in the LDAEP marker of central serotonin function. This result indicates that in this small sample of healthy control participants, pindolol does not augment central serotonin function increases to citalopram. While the findings are preliminary and are limited by the unavailability of data on the occupancy of 5-HT_{1A} receptors by pindolol, the lack of an effect on central serotonin function may provide further support for why pindolol may not be an effective therapeutic strategy to augment serotonin function and antidepressant response.

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