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ORIGINAL INVESTIGATION

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Acute effects of venlafaxine and paroxetine on serotonergic transmission in human volunteers

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Abstract *Rationale:* Antidepressant drugs are thought to enhance serotonergic neurotransmission through postsynaptic 5-HT_{1A} receptors. This effect is delayed in animals and may be paralleled by a delay in the onset of a clinical response in humans. In humans, the growth hormone (GH) response to intravenous L-tryptophan (IV L-TRP) is blocked by the 5-HT_{1A} antagonist pindolol and the prolactin response is blunted. Both are therefore thought to be a useful measure of 5-HT_{1A} receptor function. Clomipramine has previously been found to enhance the GH and prolactin responses to IV L-TRP after only 2 h. *Objective:* The purpose of this study was to use this method to investigate the effects of newer antidepressants on 5-HT_{1A} receptor-mediated function. *Methods:* Twelve healthy male volunteers took part in a random order, double blind study, in which 18.75 mg venlafaxine, 5 mg paroxetine or placebo was administered 3 h before infusion of L-TRP. *Results:* Pretreatment with venlafaxine significantly enhanced the growth hormone (GH) response to the infusion compared with pretreatment with placebo. There was no significant difference between the GH response following paroxetine compared with placebo or with venlafaxine. *Conclusions:* The data suggest enhancement of transmission through postsynaptic 5-HT_{1A} receptors by venlafaxine but not paroxetine, after only 3 h.

Key words L-Tryptophan · Receptor · Serotonin · Growth hormone · Prolactin · Human volunteer · Venlafaxine · Paroxetine

Introduction

The GH response to the intravenous infusion of L-TRP appears to be a useful measure of postsynaptic 5-HT_{1A} receptor function in man (Smith et al. 1991) and has consistently been shown to be blunted in depressive illness (Power and Cowen 1992). This abnormality appears to resolve following successful treatment (Upadhyaya et al. 1991) and there is evidence that specific antidepressant medications enhance 5-HT_{1A} receptor function in both healthy volunteers and depressed subjects (Price et al. 1990). However, the time course of this enhancement and its relationship to the onset of antidepressant action is not clear.

It has been suggested that delay in the onset of clinical response in antidepressant therapy may be due to a delay in the increase in transmission through postsynaptic 5-HT_{1A} receptors and that this delay is due to the influence of negative feedback mediated by somatodendritic 5-HT_{1A} receptors (Blier and de Montigny 1994). This conflicts, however, with the results of Anderson and Cowen (1986) which show that the tricyclic antidepressant clomipramine increases the GH response to IV L-TRP only 2 h after pretreatment in healthy volunteers. The acute effects of other antidepressants on neuroendocrine responses to IV L-TRP have not, however, been investigated in humans.

We sought to investigate the acute effects on the GH response to IV L-TRP of two newer antidepressant agents with more selective profiles than clomipramine: paroxetine, a selective serotonin re-uptake inhibitor and venlafaxine, a specific serotonin and noradrenaline reuptake inhibitor.

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Materials and methods

Subjects and experimental design

Twelve healthy male volunteers, aged 18–40 years (mean 28.1, SD 5.7), gave their informed consent to the study which was approved by the local Ethics Committee. They had no history of significant psychiatric or physical illness and had been on no medication for at least 2 months.

Subjects were tested on three occasions, at least 3 weeks apart, having taken pretreatment medication at 0700 hours on the morning of testing. Pretreatment medication consisted of placebo, par-

oxetine 5 mg or venlafaxine 18.75 mg administered orally in a balanced order, double-blind, cross-over design. Following an overnight fast, subjects attended the research laboratory at 0900 hours, when an intravenous cannula was inserted. This was kept patent with heparinised saline. Subjects fasted throughout the experiment, remained semi-supine and were not allowed to sleep. After 1 h, an infusion of L-TRP (in aqueous solution 10 g/l) was given, at a dose of 100 mg/kg, over 25 min. Blood samples were taken every 15 min from 30 min before the infusion (–30 min, –15 min and time 0) and every 15 min from 5 min until 95 min after completion of the infusion (+5 min, +20 min etc.).

Biochemical measures

Blood samples were taken into EDTA tubes and centrifuged to remove plasma. This was stored at –20°C. Plasma was also ultra-filtered and stored until assay. Samples were analysed for prolactin, GH and cortisol by standard radioimmunoassay. Free TRP was measured using high performance liquid chromatography (Marshall et al. 1987). Intra- and inter-assay coefficients of variation for prolactin were 5.7% and 6.4%, respectively, for GH 2.6% and 7.4% and free TRP 3.4%, 4.4% and total TRP 3.3% and 4.4%.

Analysis

SPSS for Windows Release 7 (SPSS, Chicago, Ill., USA) was used for statistical analysis. In all cases the Kolmogorov-Smirnov test was used to exclude any significant departure from a normal distribution. The biochemical and hormonal data were analysed using a two-way repeated measures analysis of variance (ANOVA), with drug (paroxetine/venlafaxine/placebo) and time as within subject variables. The reported *P* values of all ANOVAs used the Huynh-Feldt correction factor when the sphericity assumption was not met. For clarity, uncorrected degrees of freedom are reported.

Hormonal responses were also analysed using the trapezoid area under the curve (AUC) method. This was measured from the average of the three baseline measures taken prior to infusion of L-TRP. AUCs and average baseline measures were analysed by one-way repeated measures ANOVA with drug as a within-subjects factor. Both AUCs and baselines were then analysed for three separate comparisons (paroxetine versus placebo, venlafaxine versus placebo and paroxetine versus venlafaxine) using post-hoc paired *t*-tests (two-tailed). These data are quoted as means±SEM.

Results

Two subjects failed to complete the second trial because of intolerance of side effects (nausea and vomiting) and because the analysis was within subjects the data from the first trial was not used (order of administration – paroxetine/venlafaxine and venlafaxine/paroxetine). One subject did not complete the third trial, when venlafaxine had been administered, (paroxetine/placebo/venlafaxine) and data are therefore missing for this trial. Data from this subject are included in the post hoc comparison of paroxetine versus placebo, but not in the repeated measures ANOVA, in which data from the remaining nine subjects are included.

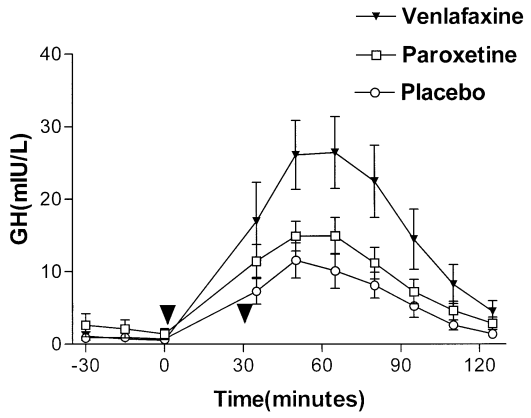

Fig. 1 Effect of pretreatment with paroxetine or venlafaxine or placebo on GH response to IV infusion of L-TRP. GH levels (mIU/l) are plotted as mean±SEM against time. The time of the infusion is indicated by *bold arrows*. ▼ Venlafaxine, □ paroxetine, ○ placebo

Growth hormone

One set of data was excluded (venlafaxine trial) because of high baseline GH values (>10 mIU/l), since GH inhibits its own secretion (Checkley 1980). This subject's baseline GH values after paroxetine and placebo were within accepted limits. ANOVA is therefore reported on data from eight subjects. ANOVA showed a significant effect of drug on GH response to L-TRP infusion (F=4.79; df=2,14; P=0.026), a significant drug by time interaction (F=2.80; df=18,126; P=0.021) and a significant effect of time (see Fig. 1 and Table 1). ANOVA of AUCs showed a significant effect of drug (F=0.038; df=2,14; P=0.038). Post-hoc analysis of AUC measures showed a significant difference between venlafaxine and placebo (venlafaxine 1676±338; placebo 598±146; t= 2.71; df=1,7; P=0.030; 95% CI 138-2019) but no significant difference between AUC for paroxetine versus placebo (paroxetine 786±214; placebo 600±117; t=0.64; df=1,9; P=0.537; 95% CI-469-842) or venlafaxine versus paroxetine (venlafaxine 1676±338; paroxetine 961±

Table 1 Effects of venlafaxine, paroxetine and placebo pretreatment on responses to L-TRP infusion: summary of analysis of variance results. Significant findings are shown in bold

	Drug			Time			Drug×time		
	df	*f*	*P*	*df*	*f*	*P*	*df*	*f*	*P*
GH	2,14	4.79	0.026	9,63	27.53	<0.001	18,126	2.80	0.021
Prolactin	2,16	2.71	0.097	9,72	8.81	0.014	18,144	2.00	0.163
Free tryptophan	2,16	0.42	0.589	9,72	122.89	<0.001	18,144	1.01	0.449

Table 2 Effect of pretreatment with venlafaxine and paroxetine on mean baseline measures and responses (calculated as trapezoid area under the curve) to L-TRP infusion for all subjects. Because of missing data for two subjects from the venlafaxine arm of the study, paired *t*-tests for comparisons with venlafaxine used mean AUCs which were different from those given in the table

		Placebo *n*=10	Venlafaxine *n*=9	Paroxetine *n*=10
Growth hormone (mIU)	AUC	600±117	1677±338[a]	786±214
	Baseline	0.86±0.24	0.79±0.33	2.04±1.17
Prolactin (mIU)	AUC	7760±3940	18120±8200	9010±3800
	Baseline	142±19	140±17	139±15
Free tryptophan (nmol/ml)	AUC	45200±3640	45600±1830	41700±2420
	Baseline	7.4±0.9	6.7±0.8	7.3±0.7

[a] *n*=8

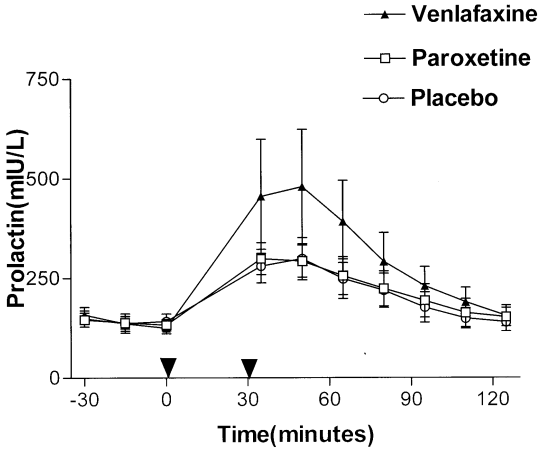

Fig. 2 Effect of pretreatment with paroxetine or venlafaxine or placebo on prolactin response to IV infusion of L-TRP. Prolactin levels (mIU/l) are plotted as mean±SEM against time. The duration of the infusion is indicated by *bold arrows*. ▼ Venlafaxine, □ paroxetine, ○ placebo

227; *t*=1.75; *df*=1,9; *P*=0.123; 95% CI –1681–250) (see Table 2). ANOVA showed no effect of drug on mean baseline values (*F*=0.29; *df*=2,14; *P*=0.653).

Prolactin

ANOVA showed no significant effects of drug or significant drug by time interaction, but a significant effect of time (Fig. 2, Table 1). There was no effect of drug on AUCs (*F*=2.02; *df*=2,16; *P*=0.172) and post-hoc comparison of AUCs showed no significant differences between the conditions (see Table 2 for mean values). There was no effect of drug on mean baseline values (*F*=0.66; *df*=2,16; *P*=0.473).

Free tryptophan

ANOVA showed a significant effect of time but no drug effect or drug by time interaction (see Table 1). There was no effect of drug on AUCs (*F*=0.36; *df*=2,16; *P*=0.625) and post-hoc comparison of AUCs showed no significant difference between the three conditions (see Table 2 for mean values). There was no effect of drug on mean baseline values (*F*=0.59; *df*=2,16; *P*=0.540).

Discussion

The main finding of the study is that pretreatment with 18.75 mg venlafaxine 3 h before infusion, significantly enhanced the GH response to infusion of L-TRP compared with placebo. There was no significant effect of paroxetine compared with placebo and no significant effect of either drug on prolactin responses.

The GH response to L-TRP has been shown to be attenuated by the 5-HT_{1A} antagonist pindolol (Smith et al. 1991). Although pindolol has β-antagonistic properties in dynamic tests of noradrenergic function (Aellig 1976), propranolol, a β-antagonist which has a much lower affinity for 5-HT_{1A} receptors than pindolol (Hoyer 1988), increases rather than decreases the GH response to L-TRP (Upadhyaya et al. 1990). This suggests that the effect of pindolol in inhibiting the GH response to L-TRP is not mediated via β-adrenoceptor blockade but probably involves 5-HT_{1A} receptor antagonism. The GH response was not attenuated by the non-selective 5-HT antagonist metergoline (McCance et al. 1987). The explanation for this may lie in a relative lack of effective antagonism of 5-HT_{1A} receptors by metergoline that has been demonstrated in functional studies in animals (Koenig et al. 1987). These findings therefore support a role for postsynaptic 5-HT_{1A} receptors in the GH response to L-TRP.

There is evidence that the PRL response to L-TRP infusion may have a dopaminergic component (van Praag et al. 1987). Pindolol causes markedly less attenuation of the PRL response than the GH response to L-TRP (Smith et al. 1991). L-TRP competes with tyramine for transport across the blood-brain barrier (Wurtman 1982) and may reduce dopamine synthesis by reducing brain tyramine. This is supported by evidence that an intravenous infusion of 5 g L-TRP causes a reduction in post probenecid cerebrospinal fluid (CSF) concentrations of the dopamine metabolite homovanillic acid (HVA) (van Praag et al. 1987). The PRL response to L-TRP may therefore be mediated in part by a reduction in dopamine synthesis, which releases PRL secretion from tonic inhibition by dopamine.

Venlafaxine is a potent serotonin reuptake inhibitor and a noradrenaline reuptake inhibitor. The active metabolite *O*-desmethyl-venlafaxine (ODV) has similar properties and both are weak dopamine reuptake inhibitors. (Muth et al. 1991). The time to maximum concentration of venlafaxine is approximately 2 h and for ODV approximately 4 h (Morton et al. 1995). At 3 h (the time between administration and infusion in this study) the concentrations of each could therefore be expected to be nearly maximal. Preliminary reports in humans suggest that at lower doses of venlafaxine (75 mg/day), there is little effect on noradrenergic reuptake (Debonnel et al. 1998). At the dose used in this study (18.75 mg), noradrenergic reuptake inhibition may not therefore be a significant factor.

This may also be the case in the study of Anderson and Cowen (1986), which demonstrated that the GH response to L-tryptophan infusion was enhanced by pretreatment with clomipramine. Clomipramine differs from venlafaxine in that it does possess anticholinergic and antihistaminergic properties (Hall and Ogren 1981). While its metabolite, desmethylclomipramine, is a potent noradrenaline reuptake inhibitor, clomipramine is not (Carlsson et al. 1969a, 1969b). At the time of infusion in the Anderson and Cowen study, little desmethylclomipramine would have been present (Jones and Luscombe 1976), suggesting that noradrenergic reuptake inhibition would be unlikely to contribute significantly to the results.

Paroxetine did not significantly increase either the GH or prolactin response to IV L-TRP. As discussed, at the doses employed, clomipramine (Anderson and Cowen 1986) and venlafaxine were probably acting primarily via effects on serotonin reuptake. It would therefore be expected that paroxetine would have similar effects. We chose dosages which were 25% of the usual daily starting dose (paroxetine 20 mg, venlafaxine 75 mg). In both cases, the starting dose is usually an effective antidepressant dose. A small dose was used because we were concerned that the side effects of the infusion would be amplified by the pretreatments. In fact, even at the low doses employed, some subjects were unable to tolerate the protocol. Clearly, a significant effect of paroxetine might have been seen at higher doses. In addition, the mean time to peak plasma concentrations of paroxetine is 5 h (Holliday and Plosker 1993). Peak plasma levels were therefore probably not reached at the time of infusion. It is notable that the GH response to IV L-TRP following venlafaxine was not significantly greater when directly compared with that following paroxetine. Using a larger number of subjects, a significant enhancement by paroxetine compared with placebo or possibly of venlafaxine compared with paroxetine, may have been shown.

The finding of a significant enhancement with venlafaxine as opposed to paroxetine at roughly equivalent doses may relate to various putative differences in their pharmacological and clinical profile. However, we believe that the noradrenaline reuptake inhibition of venlafaxine is unlikely to be a major factor at the dose employed. It has been suggested that venlafaxine may produce an earlier onset of clinical response than other antidepressants (Montgomery 1995) and that this may relate to early downregulation of βb adrenoceptors (Moyer et al. 1992). However, more recent research suggests that early β-adrenoceptor downregulation may only occur in the pineal gland, which lacks serotonergic innervation, and that generally venlafaxine does not produce this effect (Nalepa et al. 1998). While venlafaxine is a potent serotonin re-uptake inhibitor in vivo (Beique et al. 1996), it has comparatively less potency than paroxetine in blocking serotonin reuptake in vitro (Bolden-Watson and Richelson 1993). A recent study in rat brain shows a relatively low binding (2000 times less than paroxetine) of venlafaxine to the 5-HT transporter. It has been suggested, therefore, that a different mechanism of functional 5-HT re-uptake inhibition may be involved (Beique et al. 1998). This might be important in determining the speed with which postsynaptic 5-HT_{1A} receptor function is enhanced.

Both L-tryptophan and L-5-hydroxytryptophan (5-HTP) have been found to depress raphé neuron firing in animals (Gallager and Aghajanian 1976). Electrophysiological studies also suggest that acute administration of SSRIs inhibit firing of serotonergic neurones (Chaput et al. 1986; Hajós et al. 1995) and a recent study shows that this is also the case for venlafaxine (Gartside et al. 1997). However, both 5-HTP (Gartside et al. 1992) and antidepressants (Fuller 1994) increase cortical extracellular 5-HT following acute treatment, an increase which is proportionally smaller in the cortex than in the raphé nuclei (Artigas 1993). It could be argued that this is secondary to an unphysiological "spilling" of 5HT from intraneuronal stores into the synapse, in the absence of cell firing. However, the acute increase in extracellular 5-HT seen following treatment with 5-HTP (Gartside et al. 1992) and antidepressants (Rutter and Auerbach 1993) is blocked by the 5-HT_{1A} autoreceptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin, which shuts down serotonin neuronal firing. The rise in extracellular 5-HT following 5-HTP also appears to be calcium dependant, further suggesting a dependence upon neuronal firing (Gartside et al. 1992). Since L-tryptophan is the precursor to 5-HTP, we would argue that the response to the infusion and its enhancement by venlafaxine are not simply due to leakage of 5-HT and inhibition of its reuptake.

Our results suggest that venlafaxine, in common with clomipramine, enhances transmission through postsynaptic 5-HT_{1A} receptors after only 3 h. Whether this effect in humans is specific to these drugs, or occurs with other classes of antidepressants and what is the exact mechanism by which this occurs, is unclear at present.

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