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Atomoxetine increases salivary cortisol in healthy volunteers

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Abstract

It has been proposed that acute hypothalamo-pituitary-adrenal (HPA) axis challenge using noradrenergic drugs may be of utility in assessing the functional integrity of central noradrenaline pathways. Atomoxetine (formerly tomoxetine) is a highly selective noradrenaline reuptake inhibitor, which has recently been licensed for the treatment of attention deficit hyperactivity disorder (ADHD).

The aim of this study was to assess the effects of acute atomoxetine on salivary cortisol levels for the first time.

A total of 60 healthy male volunteers received 60 mg atomoxetine, 30 mg citalopram, or placebo *per os* in a double-blind parallel groups design (*n*=20 per group). Salivary cortisol, blood pressure and pulse rates were recorded at baseline and at +1.0, +1.5, +2.5 and +3.5 hours after capsule administration.

60 mg atomoxetine led to highly significant increases in salivary cortisol and a moderate increase in pulse rate, in the absence of significant effects on blood pressure. 30 mg citalopram had no significant effects on cortisol or cardiovascular parameters.

These data support the utility of atomoxetine neuroendocrine challenge for evaluating central noradrenaline pathways, which may be of future use in neuropsychiatric patient studies. Furthermore, the effects of atomoxetine on HPA axis function may have clinical implications given the use of this agent in the treatment of ADHD.

Keywords

ADHD, hyperactivity, impulsivity, neuroendocrine, HPA, noradrenaline, depression

Introduction

Acute pharmacological potentiation of the noradrenergic system in healthy volunteers, using drugs such as desipramine (a mixed reuptake inhibitor) and reboxetine (a selective noradrenaline reuptake inhibitor), have been shown to increase circulating levels of hypothalamo-pituitary-adrenal (HPA) axis hormones including adrenocorticotrophic hormone (ACTH) and cortisol (Nutt *et al*., 1987; Schule *et al*., 2004). Noradrenaline is thought to augment HPA axis function by increasing corticotrophin releasing hormone (CRF) at the paraventricular nucleus of the hypothalamus (Plotsky, 1987; Curtis *et al*., 2002; Hill *et al*., 2003). As such, it has been proposed that neuroendocrine challenge using noradren-ergic drugs may represent a useful method for probing the functional integrity of central noradrenergic pathways (Hill *et al*., 2003). Salivary cortisol sampling has been developed as a simple non-invasive convenient proxy marker of central HPA axis function, and has been shown to be sensitive to noradrenergic manipulation with reboxetine (Hill *et al*., 2003). Moderate to high correlations have been reported between salivary and serum cortisol in several populations, including healthy volunteers and depressive patients (Kirschbaum and Hellhammer, 1994; Goodyer *et al*., 1996). Some studies suggest that salivary cortisol assessment may be more sensitive to HPA manipulations than plasma cortisol assessment (Lo *et al*., 1992; Aardal-Eriksson *et al*., 1998).

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Atomoxetine has recently been licensed for the treatment of ADHD (Stahl, 2003; Biederman *et al*., 2004; Thomason and Michelson, 2004; Faraone *et al*., 2005), and represents the most selective reuptake inhibitor for the noradrenaline transporter currently available for human use. The present study examined the effects of acute atomoxetine on salivary cortisol levels in healthy male participants. We also quantified the effects of acute citalopram (a selective serotonin reuptake inhibitor), as serotonin has also been implicated in HPA axis regulation (Nadeem *et al*., 2004). It was hypothesized that noradrenergic potentiation with atomoxetine would increase salivary cortisol levels.

Methods

Subjects

Sixty healthy male volunteers (mean age [+/– SD] = 25.73 [4.70] years; range 20–35 years) were recruited using adverts in the local community, and were entered into the study on the basis of meeting inclusion criteria and providing written informed consent. Criteria for inclusion were no significant history of psychiatric or medical illnesses or recreational drug dependency (for full description of criteria please see online supplementary section for Chamberlain *et al*., 2006).

Procedure

Prior to enrolment, medical screening was undertaken by a psychiatrist to ensure there were no contraindications. Participants were asked to have breakfast as usual on the day of the study but to abstain from caffeine and alcohol. Written informed consent was obtained, and volunteers ingested a single capsule (containing 60 mg atomoxetine, 30 mg citalopram or placebo) with water in a between-subjects double-blind parallel groups design. These doses were selected to be clinically significant, i.e. towards the upper end of the usual daily dosing range for psychiatric illnesses in the United Kingdom (British National Formulary guidance). Larger doses were avoided due to their association with increased risk of discomfort and adverse events in volunteers (see e.g. Lile *et al*., 2006). After dosing, volunteers then spent 1.5 hours relaxing (watching TV or reading) in a quiet waiting area, followed by neuropsychological assessment (neuropsychological data reported previously, see Chamberlain *et al*., 2006). Blood pressure was recorded by nursing staff using electronic apparatus, and participants provided salivary samples, at baseline and at +1.0, +1.5, +2.5 and +3.5 hours after capsule ingestion.

Salivary analysis

Saliva samples were taken from volunteers using the Sarstedt Salivette™ System, centrifuged, and stored at –20 degrees Celsius prior to analysis. Analysis was conducted by the Department of Anatomy, University of Cambridge, using established procedures (Harris *et al*., 2000) (full description of the assay procedure is available from the authors on request).

Statistical analyses

Data for cardiovascular and cortisol measures were analysed using repeated-measures ANOVAs. Where significant group by time interactions or significant effects of group were found, these were investigated using one-way ANOVAs at each time point, and follow-up pairwise least significant difference (LSD) tests as appropriate (atomoxetine-treated group versus placebo group; citalopram-treated group versus placebo group). Total area under the concentration–time curve minus baseline (AUC) and the largest change in cortisol since baseline readings (delta cortisol max) were also recoded for each volunteer. These indices were subjected to one-way ANOVA. Significance was set at $p < 0.05$.

Results

The three groups were well matched for age, education levels, verbal IQ estimates (National Adult Reading Test, Nelson, 1982), and depressive mood scores (Beck Depression Inventory, Beck and Beamesderfer, 1974) (data reported in Chamberlain *et al*., 2006, supplementary online section). The groups did not differ significantly in terms of the time of capsule administration (mean time of capsule dosing [+/– SD mins], atomoxetine 9.46 AM [46], citalopram 9.50 AM [57], placebo 10.21 AM [86] (ANOVA $(2,57) = 1.374$, $p = 0.26$).

Atomoxetine and citalopram were in general very well tolerated by participants. One volunteer became transiently nauseous approximately 0.5 hours after capsule, but this resolved with a glass of orange juice and the participant was happy to complete the study. The volunteer reported that he had not taken his usual breakfast prior to attending. Code break after the study had been completed (i.e. after $n = 60$ participants were tested) showed that this volunteer had received atomoxetine.

Data for pulse rate, systolic blood pressure and diastolic blood pressure (Fig. 1) were entered into three separate repeated-measures ANOVAs with time as the within-subjects factor. For pulse rate, there was a significant effect of time $(F(4,228) = 30.432$, $p < 0.001)$ and a significant group by time interaction $(F(8,228) = 3.563$, $p < 0.001)$. From Fig. 1 it can be seen that the atomoxetine-treated group showed increasing pulse rate as a function of time compared to other groups. There were no differences in pulse rate at baseline $(F(2,57) = 0.099$, $p = 0.906)$, nor at +1.0 h $(F(2,57) = 1.183$, $p = 0.314)$ and +1.5 h after capsule $(F(2,57) = 2.107$, $p = 0.131)$. However groups did differ significantly at +2.5 h $(F(2,57) = 3.425$, $p = 0.039)$ and +3.5 h after capsule $(F(2,57) = 3.852$, $p = 0.027)$, due to significantly higher pulse rates in the atomoxetine-treated group versus placebo (least significant LSD $p = 0.031$). The citalopram group did not differ from placebo on these measures (most significant LSD $p = 0.586$). For systolic blood pressure, there were no significant effect of time $(F(4,228) = 1.941$, $p = 0.104)$, no significant group by time interaction $(F(8,228) = 0.436$, $p = 0.899)$ and nor was there a significant effect of group $(F(2,57) = 0.981$, $p = 0.381)$. For diastolic blood pressure there was a significant effect of time $(F(4,228) = 4.940$, $p < 0.001)$, but there was no significant group by
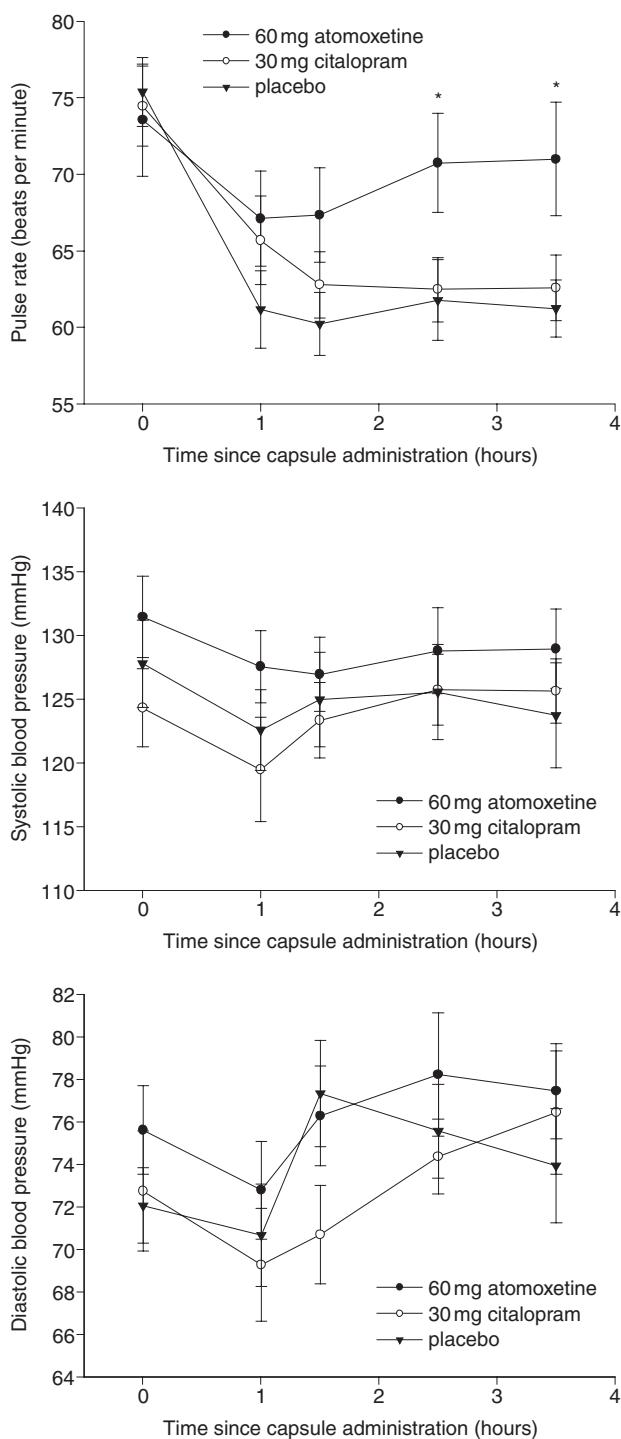
Figure 1 The effects of atomoxetine and citalopram on cardiovascular measures
Note
*$p < 0.05$ atomoxetine-treated group showed significantly higher pulse rate than placebo (LSD tests following significant ANOVA). Error bars refer to SEM.

time interaction ($F_{(8,225)} = 1.225$, $p = 0.285$) nor was there a significant effect of group ($F_{(2,57)} = 1.711$, $p = 0.190$).

Data for salivary cortisol levels (Fig. 2) were analysed using a repeated-measures ANOVA with time as the within-subjects factor. Several data points were missing due to insufficient saliva volumes for chemical analysis. Where possible these missing points were estimated by taking the mean of the two salivary cortisol levels either side of the reading time-wise ($n = 4$ of 360 samples). Otherwise, points were entered as missing data into the ANOVA model ($n = 19$ of 360 samples). There was a significant effect of time ($F_{(4,204)} = 5.080$, $p < 0.001$) and a significant group by time interaction ($F_{(8,204)} = 4.534$, $p < 0.001$). The groups did not differ significantly in terms of baseline salivary cortisol ($F_{(2,52)} = 2.420$, $p = 0.099$), nor for salivary cortisol at $+1$ h ($F_{(2,53)} = 2.671$, $p = 0.078$). Groups did differ overall for salivary cortisol levels at $+1.5$ h ($F_{(2,54)} = 4.361$, $p = 0.018$), $+2.5$ h ($F_{(2,53)} = 6.779$, $p = 0.002$), and $+3.5$ h ($F_{(2,52)} = 8.577$, $p < 0.001$). *Post hoc* tests showed that these overall group differences were attributable to higher cortisol levels in the atomoxetine-treated group compared to the placebo group at all three time points (least significant LSD $p = 0.005$). The citalopram-treated group did not differ from the placebo group for salivary cortisol levels at these time points (most significant LSD $p = 0.112$). In the total area under the curve (AUC) analysis, there was a significant overall difference between the groups ($F_{(2,51)} = 7.963$, $p < 0.001$). *Post hoc* tests showed the atomoxetine-treated group had significantly larger AUC than the placebo group (LSD $p < 0.001$) whereas the citalopram group did not differ significantly from placebo (LSD $p = 0.274$). In the delta cortisol max analysis, the groups differed in the ANOVA ($F_{(2,52)} = 11.095$, $p < 0.001$). *Post hoc* tests revealed that the atomoxetine-treated group showed a significantly larger delta cortisol max than the placebo group (LSD $p < 0.001$) whereas the citalopram-treated group did not differ from the placebo group on this measure (LSD $p = 0.644$).

Discussion

Acute dosing with 60 mg atomoxetine led to clear increases in salivary cortisol in healthy male volunteers (total AUC and delta cortisol max $p < 0.001$ versus placebo). The time course of this response corresponded approximately to the established pharmacokinetic profile of this drug, which shows peak plasma concentrations 1–2 h after oral dosing (Sauer *et al*., 2005) (Fig. 2). These findings are consistent with previous acute studies using the less selective noradrenaline reuptake inhibitor reboxetine (Hennig *et al*., 2000; Schule *et al*., 2004). Atomoxetine also increased pulse rate in the absence of significant effects on blood pressure. It will be important in future work to assess longer term effects of atomoxetine. Schule and colleagues reported recently that 5-week treatment with reboxetine in depressed patients was associated with reductions in HPA axis function, assessed using the dexamethasone suppression/corticotropin releasing hormone test. Mirtazapine, an antagonist of noradrenergic and serotonergic sub-receptors, exerted more complex effects (Schule *et al*., 2004; Schule *et al*., 2006). Consequentially, it is likely but has yet to be
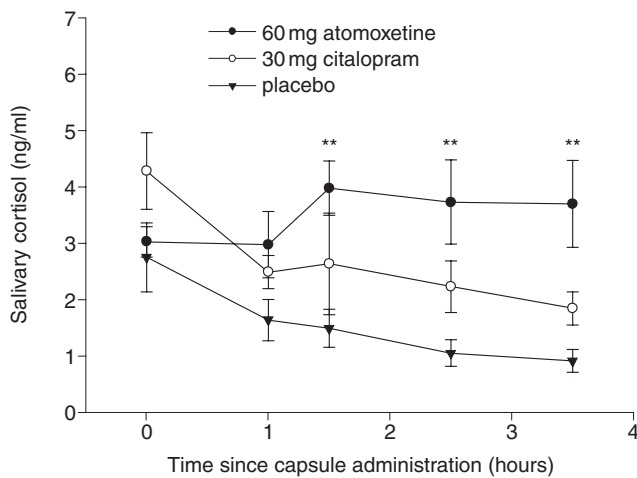
Figure 2 The effects of atomoxetine and citalopram on salivary cortisol levels

Note

**$p < 0.01$ atomoxetine-treated group showed significantly higher cortisol levels than placebo (LSD tests following significant ANOVA). Error bars refer to SEM.

demonstrated that the effects of atomoxetine on the HPA axis may differ between healthy volunteers and patients, and between acute and chronic treatment interventions.

Contrary to our expectations, we did not find a significant effect of 30 mg oral citalopram treatment on salivary cortisol. In prior healthy volunteer studies, a lower dose of oral citalopram (20 mg) was found to significantly increase salivary cortisol (Nadeem *et al*., 2004), and intravenous citalopram (20 mg) was found to increase plasma cortisol (Seifritz *et al*., 1996). Discrepancies between studies may relate to different participant characteristics (we included only male participants whereas the study of Nadeem and colleagues included both men and women) or different assessment methods (we used salivary cortisol, whereas Seifritz *et al*. used plasma cortisol measurements). Alternatively, it may be that the effects of 30 mg citalopram on cortisol in the present study were confounded or masked by the baseline difference that was evident for citalopram-treated versus placebo groups on salivary cortisol levels (see time +0 h, Fig. 2). Potential limitations of the present study include enrolment of only male participants, and that waking times of participants were not assessed. We included only male participants to maximize sample homogeneity and because prior work indicated greater responsiveness of the HPA axis to noradrenergic manipulation in males compared to females (Tse and Bond, 2005). Many patients with anxiety and depression are female (Chamberlain and Sahakian, 2004) and therefore it would be of value also to assess the effects of atomoxetine in females. This study assessed salivary cortisol, but it is important to highlight the recent development of alpha-amylase as a peripheral marker for central noradrenaline function that could be measured in future work (van Stegeren *et al*., 2006).

In sum, this study reported increases in salivary cortisol, a marker of HPA axis function, following acute treatment with atomoxetine in healthy volunteers. Evaluation of the effects of atomoxetine on HPA axis function in depression and ADHD would be of value, to assess the integrity of the noradrenergic system in these disorders, and to help elucidate the mechanisms by which noradrenergic drugs are able to exert their beneficial treatment effects (Sheline, 2000; Varghese and Brown, 2001; Goodyer *et al*., 2003; Chamberlain and Sahakian, 2005).

Acknowledgements

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