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Effects of antipsychotics on cortisol, interleukin-6 and hippocampal perfusion in healthy volunteers

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ABSTRACT

This randomized within-subject, double blind study aimed to compare the effects of a single dose of two different antipsychotics (haloperidol and aripiprazole) on cortisol, interleukin (IL)-6 and hippocampal regional Cerebral Blood Flow (rCBF) in the same 17 healthy male individuals. Subjects received a single dose of haloperidol (3 mg), aripiprazole (10 mg) and placebo, in a randomized order on three study appointments. We measured salivary cortisol levels at multiple time points, IL-6 levels from plasma samples, and resting cerebral blood flow (rCBF), using a pulsed continuous arterial spin labeling (pCASL) sequence (1.5T). We found significantly lower cortisol levels in the haloperidol condition ($F(2,32) = 5.78$, $p = 0.007$), than in either placebo ($p = 0.013$; CI = 0.45, 0.406) or aripiprazole ($p = 0.037$; CI = −0.520, −0.014). Interleukin-6 levels were also lower following haloperidol ($F(2,22) = 4.19$, $p = 0.048$) in comparison with placebo ($p = 0.02$; CI = 0.14, 1.8), but not with aripiprazole. Finally, we found a greater rCBF in the right (peak voxel: $T = 6.47$, $p < 0.0001$) and left (peak voxel $T = 5.17$, $p < 0.01$) hippocampus following haloperidol compared with placebo, and at trend level also in the left hippocampus following aripiprazole compared with placebo ($T = 4.07$, $p = 0.057$). These differences in hippocampal rCBF after both antipsychotics were no longer evident (haloperidol) or present at trend level (aripiprazole), after controlling for cortisol and IL-6 levels. Our findings suggest that haloperidol can directly regulate the hypothalamic-pituitary-adrenal (HPA) axis and immune system through a pharmacological action via D2 receptor antagonism. Finally, our data suggest that the increased hippocampal rCBF is a manifestation of the reduction in IL-6 and cortisol which follows the administration of haloperidol.

1. Introduction

Over the past few decades, an increasing number of studies have reported a hyper-activation of the hypothalamic-pituitary-adrenal (HPA) axis and of the immune system in patients with psychosis. These two biological systems are responsible for the main brain and behavioural changes occurring in response to stress, as well as for our defence against external pathogens. Of note, both the HPA axis and the immune system have been shown to contribute to some of the abnormalities in brain structure and function found at psychosis onset (Borges et al., 2013; Mondelli et al., 2011; Zajkowska and Mondelli, 2014), and have been proposed as optimal targets for developing new treatments for psychosis. However, it remains unclear if and how HPA axis and the immune system are affected by antipsychotic medications, and indeed if the effect of antipsychotics on the brain is partly mediated by the modulation of these biological systems.

Elevated levels of cortisol, the final hormone produced by the activation of the HPA axis, and of inflammatory markers, such interleukin (IL)-6, have been consistently shown in subjects with psychosis (even prior to treatment) and in non-psychotic at-risk individuals, indicating that the activation of these biological systems partly predate the onset of psychosis (Aiello et al., 2012; Borges et al., 2013). A dysregulation of these systems may result from prolonged exposure to psychosocial stress, and represent the biological mediator underpinning the relationship between stressful events (for example in childhood) and the development of psychotic symptoms (Walker and Diforio, 1997).

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Although the mechanisms through which increased HPA axis activity and inflammation lead to the onset of psychotic symptoms remain unknown, multiple pathways modulating monoaminergic systems and synaptic plasticity have been proposed. Interestingly, both cortisol and IL-6 directly interact with the hippocampus, a brain region consistently reported as altered in structure, function and activity in psychosis (Tamminga et al., 2010), suggesting that stress hormones and pro-inflammatory markers play a key role in mediating some of the key physiological brain changes observed in this condition. Indeed, elevated levels of both cortisol and IL-6 have been related with smaller hippocampal volumes in healthy subjects, as well as in subjects with first episode psychosis (Marsland et al., 2008; Mondelli et al., 2011).

We have previously reported that some of the structural and functional brain changes observed in psychosis are related to antipsychotic treatment (Dazzan et al., 2005; Goozee et al., 2015; Navari and Dazzan, 2009), and that the mechanisms underpinning these changes might partly depend on alterations in resting cerebral blood flow (rCBF) (Goozee et al., 2014; Handley et al., 2013). In particular, in a previous study in healthy volunteers, we showed that a single dose of either haloperidol or aripiprazole affects rCBF in the same brain regions which have been reported as structurally and functionally altered in psychosis (Handley et al., 2013). However, the mechanisms through which antipsychotic treatment affects rCBF remain unclear. Only very few human studies have linked cerebral perfusion and cortisol levels, with most data coming from animal studies. Interestingly, increased CBF in hippocampus (and to a lesser degree in the prefrontal cortex) has been described in adrenalectomized rats (Endo et al., 1994), while reduced hippocampal rCBF has been reported after 12 weeks of corticosterone administration (Endo et al., 1997) or a similar period of stress exposure in rats (Endo et al., 1999). Similarly, studies in humans mostly report an inverse correlation between cortisol levels and cerebral perfusion in the medial temporal lobe and anterior cingulate cortex (Bonne et al., 2003; Hodkinson et al., 2014). Furthermore, the inflammatory cytokine IL-6 has been reported as a potent vasoconstrictor (Tarkowski et al., 1995) and antibodies against IL-6 attenuate post-hemorrhagic vasospasm (Bowman et al., 2004), possibly leading to a decrease in cerebral perfusion.

Evidence from our and other groups suggests that the levels of both cortisol and IL-6 are affected by antipsychotic medications, which possibly re-establish 'normal' levels of these biomarkers and their regulatory processes (Miller et al., 2011; Mondelli et al., 2010a). However, it remains unclear whether this reflects a direct effect of these drugs on the stress response system, or rather an indirect effect of the amelioration of psychotic symptoms induced by these drugs. Furthermore, it is yet to be established whether antipsychotics with different pharmacological profiles affect these stress response markers, and any potentially associated brain change, differently.

Both first (FGA) and second (SGA) generation antipsychotics have been associated with cortisol changes in patients with psychosis, but increasing evidence suggests that SGAs reduce cortisol to a greater extent than FGAs (Jakovljevic et al., 2007; Popovic et al., 2007; Tanaka et al., 2008; Zhang et al., 2005). Most direct comparisons of the effects of FGAs and SGAs on stress markers have been conducted in patient populations, with only one study conducted in a small sample of healthy volunteers (Cohrs et al., 2006). This study demonstrated no effect of the dopaminergic antagonist haloperidol on cortisol levels, and a reduction on cortisol levels induced by SGA antipsychotics like quetiapine and risperidone. The effect of SGAs on cortisol has been suggested to reflect differences in affinity and occupancy at the D2 and 5-HT receptor subtypes (Meltzer, 1989).

Data on the effects of antipsychotics on IL-6 appear less consistent (Baumeister et al., 2016). For example, in patients, FGAs and SGAs have been found to increase, reduce or have no effect on IL-6 levels (Maes et al., 1995; Tourjman et al., 2013; Zhang et al., 2005). Two recent meta-analyses also reported conflicting results, indicating no significant effect of antipsychotic treatment on IL-6 (Tourjman et al., 2013) in one study, and a reduction of IL-6 after antipsychotic treatment in another (Miller et al., 2011). In one in vitro investigation in healthy females, IL-6 levels remained unchanged after both first and second generation antipsychotics (Himmerich et al., 2011). However, to the best of our knowledge, the effects of FGA or SGAs on IL-6 in healthy volunteers (in vivo) have never been investigated. Furthermore, no evidence exists, from either patients or healthy individuals, on the effects of a "third" generation antipsychotic such as aripiprazole on cortisol or IL-6.

Given that antipsychotics directly affect cortisol and IL-6, and that there is an association between these markers and cerebral blood flow, it is possible that the effect of antipsychotics on brain structure and blood flow is partly mediated by these biomarkers. Understanding the differential effects of FGAs and SGAs will help clarify whether the level of antagonism at D2, or other neurotransmitter receptors, such as the serotonin, are important in regulating cortisol and IL-6 levels, and whether these biomarkers mediate the drug-induced changes evident in hippocampal rCBF.

This study compared the effects of two antipsychotics with very different mechanisms of action: haloperidol, a strong D2 receptor antagonist with comparatively lower affinity for other receptors, such as serotonin 5-HT2 (Meltzer, 1989), and aripiprazole, a partial D2 and 5HT1A receptor agonist with a lower affinity for the serotonin 5-HT2A than for the dopamine D2 receptor subtype (Mamo et al., 2007). We compared the effects of a single dose of these two drugs on cortisol, IL-6 and hippocampal rCBF in the same healthy individuals in comparison with placebo. This is the first investigation of aripiprazole and haloperidol (in vivo) effects on cortisol and IL-6. Moreover, it is the only exploration of the effects of different antipsychotics on cortisol, IL-6 and hippocampal perfusion in the same individuals. Amongst the various pro-inflammatory cytokines which have been reported increased in psychosis, we focussed in particular on IL-6 as this remains the main pro-inflammatory cytokine which has been reported as elevated in patients with psychosis, and as being linked to activation of the HPA axis. We hypothesised that i) aripiprazole but not haloperidol would reduce cortisol; ii) both antipsychotics would reduce IL-6 levels and iii) reductions in these biomarkers would account for some of the rCBF change evident in the hippocampus following administration of these antipsychotics.

2. Methods

Seventeen healthy right-handed English speaking Caucasian males, aged 18 to 32 (mean 22 years, SD 4.1) provided salivary cortisol samples across the day and underwent a brain scan for the investigation of resting blood flow (rCBF). Twelve of them also provided blood samples for the measurement of IL-6 levels. Mean body mass index was within the normal range (mean 23.5, SD 3.7). Participants were non-smoking, university students with no recent or current drug or medication use and had no exposure to psychotropic medication, or history of personal or familial psychiatric diagnosis. The study was approved by the Human Research Ethics Committee of the Institute of Psychiatry, London, and conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants after the nature of the experimental procedures was explained to them.

Subjects received a single dose of haloperidol (3 mg), aripiprazole (10 mg) and placebo, in a randomized order on three study appointments. A fully counterbalanced, randomized within-subject, double blinded cross-over design was used, ensuring neither participant or researcher were aware of the intervention administered on each appointment. Compounds were administered in identical capsules. Fourteen days minimum separated each study appointment to allow for drug washout. No alcohol or medications were used for 24 h, or caffeine for 6 h, prior to scanning.

Clinical side effects, measured using the Barnes Akathisia scale (Barnes, 1989), the Simpson Angus scale (Simpson and Angus, 1970), and the Abnormal Involuntary Movement Scale (AIMS: National Institute of Mental Health), were evaluated 3 hours post intervention.

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Haloperidol and aripiprazole induced more extra-pyramidal side effects than placebo ($\chi^2 = 7.3$, $p < 0.05$). There were no significant differences across intervention in tardive dyskinesia ($\chi^2 = 2.0$, n.s.), akathisia (all participants had a negative score), systolic ($F(2,32) = 2.16$, not significant) or diastolic ($F(2,32) = 0.15$, not significant) blood pressure.

2.1. Salivary cortisol and interleukin (IL)-6 assessment

Using salivary cortisol salivettes (Sarstedt, Leicester, UK) subjects chewed on a cotton roll for 2 min per sample under observation, at three time points including baseline (pre-intervention, morning), 1.45 h (post intervention, early afternoon), and 3.10 h (post intervention mid-afternoon). The last sample was acquired 20 min prior to the brain scan. Salivettes were frozen at −20 °C. After thawing, they were centrifuged at 3500 rpm for 10 min, which resulted in a clear supernatant of low viscosity. Saliva cortisol concentrations were determined using the "Immulite"—Siemens Immunoassay analyzer (www.diagnostics.siemens.com). The plasma cortisol assay of the analyzer was suitably modified and then validated for these measurements. A set of 10 cortisol standards in saline were used in each assay to plot a calibration graph. This was highly reproducible with slope of (mean ± SEM) 0.197 ± 0.004. The method had analytical sensitivity of 0.2 nmol/l and inter/intra assay precision (% CV) of <10% (cortisol concentration range 5 to 25 nmol/l). All samples from the same subject were analyzed in the same run.

For IL-6, one 4 ml EDTA tube was collected 3.20 hours post intervention (10 min prior to the rCBF measurement). Samples were stored in a refrigerator (4°C) until the end of the testing day. Samples were later (approximately 3.10 h after sample acquisition) centrifuged at 1600 rpm for 10 min to allow for serum extraction which was then frozen at −80 °C. On study completion, plasma samples were unblinded and outsourced to the Department of Clinical Biochemistry, King's College Hospital London. Quantitative sandwich enzyme linked immunoassay (ELISA) analysis technique was conducted using a Quantikine IL-6 ELISA kit distributed by R & D Systems (Abingdon).

2.2. Resting blood flow (rCBF) acquisition

Participants underwent a 6 minute resting scan with eyes open and images were acquired in a General Electric Signa HDX 1.5 tesla scanner at the Centre for Neuroimaging Sciences, Institute of Psychiatry. rCBF measurements were made using a pulsed-continuous arterial spin labeling technique (see Handley et al., 2013 for full imaging technique, acquisition and image pre-processing details). Two additional structural, high spatial resolution images were acquired for co-registration and normalisation, including a T2 weighted fast spin echo and fluid-attenuated inversion-recovery fast spin echo (FLAIR FSE) scan to exclude the presence of any brain pathology.

2.3. Data analysis

Investigation of the cortisol and IL-6 data was conducted using the Statistical Package for Social Sciences, Version 15.0 (SPSS Inc.). Extreme outliers were investigated from variables for which normality assumptions (examined with the Shapiro-Wilk test) were violated and natural logarithm was applied where normality was violated, but no extreme outliers were identified. The Area Under the Curve (AUC) for cortisol levels across the three time points was calculated based on the trapezoid formula (Pruessner et al., 2003). We first explored the effects of intervention on cortisol AUC and IL-6 levels using a repeated measures analysis of variance (ANOVA), including intervention as the within-subjects factor, followed by Bonferroni corrected post-hoc comparisons.

Building on our previously published work demonstrating a significant increase in hippocampal rCBF after haloperidol and aripiprazole relative to placebo (Handley et al., 2013), we examined the role of cortisol and IL-6 on these regional rCBF changes. Using Statistical Parametric Mapping (SPM, version 5, www.fil.ion.ucl.ac.uk/spm), we conducted paired samples t tests comparing haloperidol and aripiprazole with placebo. We first confirmed that differences in hippocampal rCBF previously identified remained evident in the sub-group with available cortisol and IL-6 data, and then conducted the analysis again controlling for cortisol and IL-6 (separately) levels. A region of interest (ROI) approach was employed, using a-priori anatomically defined masks of left and right hippocampus created using the Anatomy toolbox extension (Eickhoff et al., 2005) in SPM5. Region of interest (ROI) voxel-level statistics were accepted at $p < 0.05$ corrected for family-wise error (FWE).

3. Results

3.1. Antipsychotic effect on cortisol levels

Prior to intervention, cortisol levels were not significantly different across intervention groups ($F(2,32) = 1.305$, not significant). After administering placebo, haloperidol and aripiprazole, the mean AUC values for cortisol were 7.42 (SD: 0.28), 7.20 (SD: 0.25) and 7.47 (SD: 0.37) nmol min/l respectively. There was a significant overall reduction in cortisol levels (measured as AUC) following haloperidol ($F(2,32) = 5.78$, $p = 0.007$; evident in Fig. 1). Post hoc comparisons confirmed this was significant in comparison to both placebo ($p = 0.013$; CI = 0.45, 0.406) and aripiprazole ($p = 0.037$; CI = −0.520, −0.014).

3.2. Antipsychotic effect on IL-6 levels

IL-6 data were acquired post intervention and available for a total of 12 subjects. After administering placebo, haloperidol and aripiprazole, the mean IL-6 levels were 1.76 (SD: 1.07), 0.80 (SD: 0.33) and 1.21 (SD: 0.74) pg/ml, respectively. This indicated significant lower IL-6 levels following haloperidol ($F(2,22) = 4.19$, $p = 0.048$), and post hoc analysis revealed this was in comparison with placebo ($p = 0.02$; CI = 0.14, 1.8) but not with aripiprazole (not significant).

Since there was a significant reduction in both cortisol and IL-6 levels following haloperidol compared with placebo, we explored whether cortisol and IL-6 levels were correlated after these interventions. There was no significant correlation between cortisol and IL-6 after haloperidol ($r = -0.459$, not significant) nor after placebo ($r = -0.303$, not significant).

3.3. Antipsychotic effect on resting cerebral blood flow (rCBF)

The first analysis confirmed our previously published findings (based on the larger dataset; (Handley et al., 2013)) that greater rCBF was evident in the right (peak voxel: $T = 6.47$, $p < 0.0001$) and left (peak voxel $T = 5.17$, $p < 0.01$) hippocampus following haloperidol

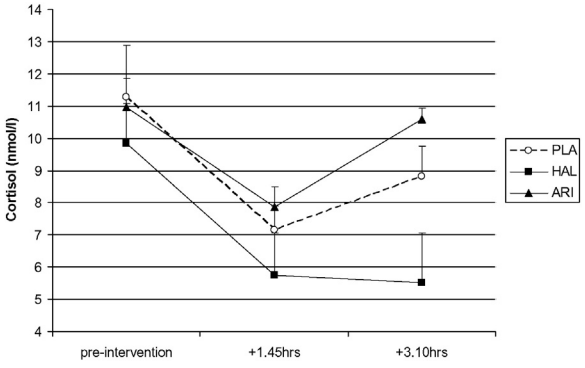

Fig. 1. Raw mean (with standard error bars) cortisol levels at individual time points: baseline (pre intervention; 10:20 am), 1.45 hours post intervention (12.15) and 3.10 hours post intervention (13:40) after placebo (PLA), haloperidol (HAL) and aripiprazole (ARI) administration.

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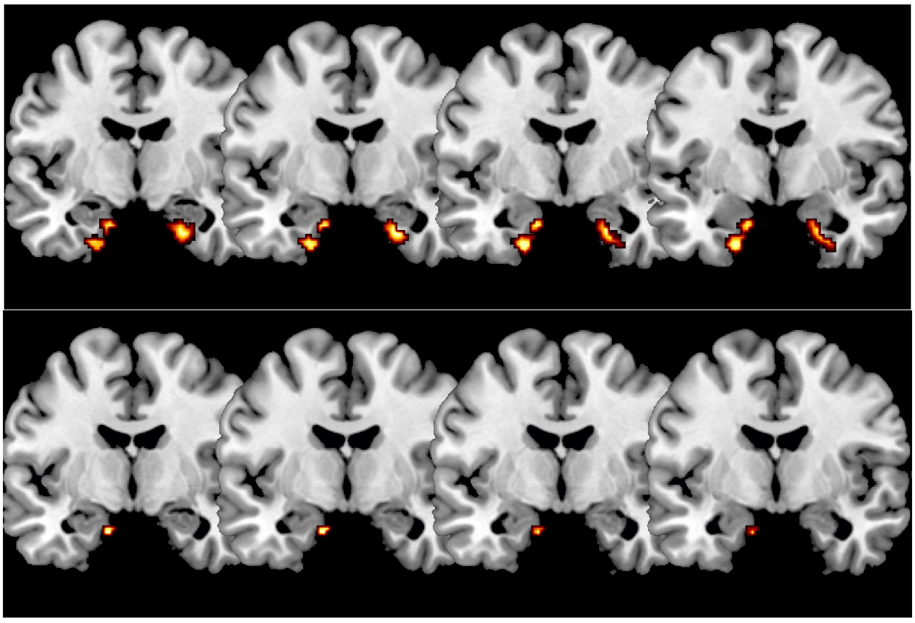

Fig. 2. Peak voxels within the hippocampus in which rCBF increased bilaterally following haloperidol (top), and on the left after aripiprazole (bottom), compared with placebo.

compared with placebo (see Fig. 2). Increased rCBF in the left hippocampus after aripiprazole compared with placebo (Fig. 2) was also evident, though the increase was only a trend in this sample ($T = 4.07$, $p = 0.057$).

We repeated this analysis to test whether these antipsychotic-induced changes in hippocampal rCBF remained evident after controlling for cortisol and IL-6 changes. We found that the differences previously identified in hippocampal rCBF after both antipsychotics were no longer significant (haloperidol), or evident at trend level (aripiprazole), suggesting cortisol and IL-6 largely accounted for the hippocampal rCBF alteration evident following antipsychotic administration.

4. Discussion

In this double-blind randomized study, we show for the first time that haloperidol, but not aripiprazole, lowers cortisol and IL-6 levels, and increases hippocampal rCBF within hours of drug administration and independently from the pathophysiology of psychosis. Our findings also provide the first novel, preliminary evidence that changes in cortisol and IL-6 levels following haloperidol administration may explain the altered hippocampal rCBF observed after administration of this antipsychotic drug.

Only one previous study has directly compared the effects of different antipsychotics on cortisol levels in healthy individuals (Cohrs et al., 2006). This group reported that cortisol levels remained unaffected by haloperidol while a significant reduction was evident following olanzapine and quetiapine (SGAs) compared with placebo. In contrast with these findings, we observed a significant reduction in cortisol levels after haloperidol, which may be due to our larger sample size ($N = 11$ vs $N = 17$), allowing greater statistical power to identify such change. It is noteworthy that Cohrs and colleagues reported an absence of haloperidol effect based on cortisol levels averaged across eight (excluding baseline) time points, and their raw data indicate that at 14:00 h, a similar time to that driving our finding after this drug, cortisol levels appeared lower than placebo. In line with our findings, another study also reported significant suppression of cortisol during laboratory induced stress conditions following acute haloperidol administration in healthy volunteers (Hennig et al., 1995). Furthermore, other compounds characterised by high D2 receptor antagonism have demonstrated similar suppressive effects on cortisol levels during stress-induced conditions (Itoh et al., 2005); consistently, FGA treatment has been associated with normalisation of previously raised cortisol levels also in patients with psychosis (Zhang et al., 2005).

The mechanisms through which haloperidol decreases cortisol levels could be related to the strong antagonistic effect of this drug on dopamine in the mesolimbic pathway, particularly in the striatum. Indeed, it has been suggested that dopamine plays a stimulatory role in the control of the HPA axis, with several D2 dopamine receptor agonists having been reported to increase plasma corticosterone levels (Borowsky and Kuhn, 1992; Foreman et al., 1989); on the other hand, a reduction in dopaminergic activity, as a result of high antagonism at the D2 receptors in the striatum, could directly lead to lowered cortisol levels (Oswald et al., 2005; Wand et al., 2007). This would suggest that the change in cortisol levels we found following haloperidol administration results directly from the pharmacodynamic effects of this antipsychotic.

In healthy volunteers, there are no studies exploring the effects of FGAs on IL-6 in vivo, however one in vitro study investigated haloperidol and chlorpromazine (both FGAs), quetiapine and clozapine (SGAs; (Himmerich et al., 2011)) in healthy females and reported no effects of any antipsychotic in toxic shock syndrome toxin TSST-1 -stimulated blood cells. Numerous methodological differences are apparent between this study and our own, not least the in vitro vs in vivo nature of investigation; moreover, it is possible that our larger sample and inclusion of a placebo condition allowed for the identification of haloperidol effects. Interestingly, haloperidol has been previously associated with reduced IL-6 levels in schizophrenia patients (Maes et al., 1995). Elevated IL-6 levels have been reported to elicit changes in dopaminergic function (Aguilar-Valles et al., 2012); conversely, our results also demonstrate that, in healthy controls, manipulation of the dopaminergic system (via haloperidol) can affect/reduce IL-6 levels. The immediacy of these effects in our study provides further evidence that these may be direct pharmacodynamic effects of haloperidol. This would, propose that changes in these markers in schizophrenia patients are unlikely to simply represent secondary drug effects resulting from reduced psychosocial stress following symptom amelioration. Interestingly, we have also recently shown that increased persistent levels of IL-6 in first episode psychosis patients are associated with poor treatment response, supporting a role of the activation of the immune system for the response to antipsychotic treatment (Mondelli et al., 2015).

The mechanisms through which haloperidol leads to a decrease in IL-6 levels are still unclear. Indeed, in contrast to the well-recognized

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immunomodulatory effects of noradrenaline and adrenaline, the influence of dopamine on inflammatory responses remains more contradictory, with differences across in vitro and in vivo studies. Also, some authors suggest that the immunomodulatory effect of dopamine might be mediated in a dose-dependent fashion by different types of receptors (Basu and Dasgupta, 2000; Beck et al., 2004). Interestingly, stimulation of D1 and D2 receptors has been described as leading to inhibition of NF-κB dependent transcription cascade, which has a key role in regulating the inflammatory response. Alternatively, the HPA axis could also partly be responsible for mediating the effects of haloperidol on the immune system. It is well known that HPA axis can affect regulation of immune system, and vice versa (Zunszain et al., 2011), partly through shared down-stream molecular pathways, such as the NF-κB; it is therefore possible that the effect of haloperidol on cortisol and IL-6 levels depends, at least partly, on shared underlying molecular pathways.

Our study is the first one to specifically explore the effects of aripiprazole on cortisol. In fact, all existing studies in patient samples grouped individuals taking aripiprazole with those taking other SGAs and did not explore the effects of this drug separately. Somehow unexpectedly, we did not find any change in cortisol levels after aripiprazole. Amongst other unique pharmacological properties that separate aripiprazole from other SGAs, this drug has a lower affinity for 5HT-2 than for D2 receptors (Mamo et al., 2007). It is interesting to note that amisulpride, the only SGA that has a similarly lower affinity for 5HT-2 than D2 receptors, has also been associated with an absence of effect on cortisol in healthy individuals (Wetzel et al., 1994), suggesting that the dopamine-serotonin affinity profile common to these drugs, and *not* other SGAs, might be important in HPA axis regulation. Together with the haloperidol effects evident in our study, this indicates that it is not only the affinity to dopamine receptors that is key in the regulation of stress and pro-inflammatory markers, but also the function of that drug on dopamine receptors (i.e. antagonistic vs partial agonism). Similarly, no change in IL-6 was observed following aripiprazole. Limited and mixed evidence for the effect of all SGAs on IL-6 makes it challenging to understand whether aripiprazole effects on pro-inflammatory markers are distinct from other SGAs, and if the lack of effect might be again related to its dopamine-serotonin affinity profile. However, for cortisol at least this study demonstrates that aripiprazole does not elicit changes comparable to those of other SGAs and future investigation should separate this antipsychotic from the SGA group.

We have previously reported (Handley et al., 2013) increased resting blood flow (rCBF) in the hippocampus following acute administration of haloperidol (bilaterally) and aripiprazole (left hippocampus), compared with placebo. In the current study we now find that accounting for individual cortisol and IL-6 levels after each intervention abolished this effect, suggesting that these biological markers may mediate, at least in part, hippocampal rCBF changes that follow the administration of these antipsychotics. Consistent with this notion, a number of studies in healthy individuals have reported elevated cortisol levels (artificially or stress induced) to be associated with lower hippocampal resting metabolism, lower right medial temporal lobe blood flow during a declarative memory task and deactivation of hippocampus during a stress task (de Leon et al., 1997; de Quervain et al., 2003; Pruessner et al., 2008). These studies suggest that higher cortisol is associated with decreased hippocampal function, and our finding that cortisol explains some of the hippocampal rCBF alterations observed after haloperidol is consistent with this hypothesis. Previous studies have reported IL-6 as a potent vasoconstrictor and have shown that long-term treatment with pro-inflammatory cytokine, such as IL-1β, decreases CBF (Maher et al., 2003), while prolonged administration of the antagonist IL-1 receptor antagonist increases CBF. This is again consistent with our finding, and suggests that a reduction in IL-6 levels could be partly responsible for the increase in hippocampal CBF following haloperidol. Interestingly, we have also recently shown that both cortisol and IL-6 are associated with reduced hippocampal volume in first-episode psychosis patients (Mondelli et al., 2011, 2010b). On the basis of our current findings, further studies would need to clarify if rCBF could be considered a plausible mediator towards the previously observed relationship between cortisol and IL-6 and structural hippocampal change in psychotic patients.

The study has a number of strengths. Notably, all three interventions were administered in a repeated measures design and cortisol, IL-6 and hippocampal rCBF were acquired in the same individuals, limiting the impact of intra-individual variability. To date, this is the largest and first study to acquire these measurements in healthy individuals allowing for the direct pharmacodynamic drug effects to be interpreted excluding the possible interaction of psychopathology.

Few main limitations of this study should be acknowledged. First, we did not measure IL-6 prior to intervention, and used placebo as the baseline measure for all individuals. However, the factors most likely to elicit cortisol and IL-6 level variations across test days are diurnal rhythm and dietary intake, and both these factors were controlled to ensure test days were identical across interventions and between individuals. Moreover, the baseline measures for cortisol were not significantly different, indicating that any factor that could potentially alter these markers prior to the test day is unlikely to have had an impact on our findings. Secondly, we chose to study the effects of these drugs on healthy individuals, to examine their direct effects on stress markers and brain perfusion. Therefore, by the very nature of our design, we cannot establish whether in clinical samples any effect of antipsychotics on stress markers and brain perfusion reflects a combined effect of underlying pathology and direct drug action. Third, we focussed our analysis only on peripheral levels of IL-6, which may give us only limited information about central inflammation; also, the analyses of other cytokines could have provided a fuller picture of the effect of antipsychotic treatment on the inflammatory response.

In conclusion, our finding that haloperidol but not aripiprazole reduces cortisol and IL-6 in healthy volunteers suggests that haloperidol could regulate HPA axis and immune system through a direct pharmacological action via D2 receptor antagonism, and independently from its effect on reducing psychosocial or symptom-induced stress. Moreover, the absence of aripiprazole effects, particularly on cortisol provides strong evidence that this antipsychotic is not directly comparable to other SGAs, highlighting the importance of investigating drug specific pharmacodynamic effects rather than groups of antipsychotic drugs. Finally, our findings suggest a role of cortisol and IL-6 in mediating rCBF changes by antipsychotic treatment, further supporting stress and inflammatory markers as viable possible targets for future antipsychotic drug development.

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Contributors

Rowena Handley and Valeria Mondelli contributed to study design, recruitment of the subjects, data collection, analysis and interpretation, and writing of the manuscript. Paola Dazzan, Carmine M. Pariante, Philip McGuire, and Antje A.T.S. Reinders, contributed to study design, analysis and interpretation of the data, and writing of the manuscript. Fernando Zelaya, Tiago Marques, Heather Taylor, and Kathryn Hubbard contributed to the recruitment of the subjects, collection of the data and writing of the manuscript. Andrew S. Papadopoulos contributed to the analysis of the cortisol samples, interpretation of the data and writing of the manuscript.

Conflict of interest

Rowena Handley is currently employed as a Medical Science Manager at Bristol-Myers Squibb Pharmaceuticals Ltd. The views expressed are those of the authors and not necessarily those of Medical Science Manager at Bristol-Myers Squibb Pharmaceuticals Ltd. Professor Pariante and Dr Mondelli have received research funding from Johnson & Johnson as part of a programme of research on depression and inflammation. In addition, Professor Pariante and Dr Mondelli have received research funding from the Medical Research

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