Neuropsychopharmacology (2009) 34, 2194–2195

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## Reply to Carroll

# Reply: 'Clomipramine and Glucocorticoid Receptor Function'

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*Neuropsychopharmacology* (2009) **34,** 2194–2195; doi:10.1038/npp.2009.43; published online 13 May 2009

Sir,

In response to Dr Carroll's letter 'Clomipramine and Glucocorticoid Receptor Function,' we are delighted that Dr Carroll has found our paper of interest and has provided comments and queries. We are confident that this reply addresses these issues satisfactorily and will help explain our data and our hypotheses better. We have shown using a whole-blood assay that the effect of antidepressants on glucocorticoid receptor (GR) function is altered in patients with major depression when compared with controls (Carvalho *et al*, 2008). These treatment-resistant depressed patients also have biological disturbances, as shown by hypercortisolemia and signs of inflammation in the blood when compared with controls (Carvalho *et al*, 2008).

The first issue raised by Dr Carroll is the lack of difference in whole-blood cells GR sensitivity to glucocorticoids between depressed patients and controls. We argue that this is still indicative of 'relative' GR resistance as depressed patients have, on average, almost twice the levels of endogenous cortisol in the '*in vitro*' assay milieu when compared with controls. Also, our conclusions are based on a very large body of evidence (reviewed in Carvalho and Pariante, 2008; Pariante and Miller, 2001) showing reduced GR function in depressed patients compared with controls. Dr Carroll cites his recent paper (Carroll *et al*, 2007) as supporting the notion that there is no problem with glucocorticoid feedback signaling in hypercortisolemic depressed patients: however, we do not assess (HPA axis) negative feedback and his paper does not assess GR sensitivity *in vitro*, so it is difficult to see how the two papers can be compared. Nevertheless, Dr Carroll offers us the helpful advice to derive a dose–response curve. We have therefore reviewed our preliminary data collected in the optimization of this assay in a small number of patients and controls. The dose–response (see Figure 1) study was conducted using an initial protocol of 4 h incubation rather than 24 h. These data clearly indicate that also in this assay depressed patients require a larger concentration of dexamethasone to inhibit GR function: the $IC_{50}$ for dexamethasone in controls is 135 nM and that in depressed patients is 776 nM. We cannot determine whether this difference is more evident using this shorter incubation time, but at least these data are consistent with the literature in showing GR resistance in peripheral blood cells of depressed patients. Chronic high glucocorticoid levels in the plasma of depressed patients (in our published sample, $429.4 \pm 55.4$ *vs* $242.2 \pm 14.8$ nM in (Carvalho *et al*, 2008) could induce GR translocation to the nucleus prior to the *in vitro* assay, and thus decrease GR function when measured. Moreover, GR function in depressed patients could be influenced by the increased levels of the pro-inflammatory cytokine, interleukin-6 (in our published sample, $3.0 \pm 0.29$ *vs* $2.4 \pm 0.1$ pg/ml in Carvalho *et al*, 2008). Indeed, cytokines produced during an inflammatory response may induce GR resistance in relevant cell types by direct effects on the GR (Pace *et al*, 2007; Miller *et al*, 2002). Indeed we have earlier shown that the pro-inflammatory cytokine interleukin 1-β inhibits dexamethasone-induced GR translocation and GR function (Pariante *et al*, 2003).

As in the original description of this method by Rohleder *et al,* (2002, 2003), the IC-50 values obtained in the dose–response curve shown in Figure 1 are indeed higher than expected, considering the affinity of dexamethasone for GR. This discrepancy is puzzling and will require further investigation. Nevertheless, considering the fact that dexamethasone-induced inhibition of IL-6 synthesis is mediated by the GR, and considering also the consistency between our results and those of earlier papers using different peripheral blood assays, we believe that the whole-blood model is a useful, biologically relevant and clinically important method to evaluate GR function.

Finally, Dr Carroll argues that the concentration of clomipramine in our whole-blood assay was toxic to our cells, and much higher than the therapeutic plasma levels obtained in patients. However, micromolar concentrations of antidepressants are achieved in the brain of animals treated with the doses used in this and similar studies

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Received 26 March 2009; accepted 26 March 2009

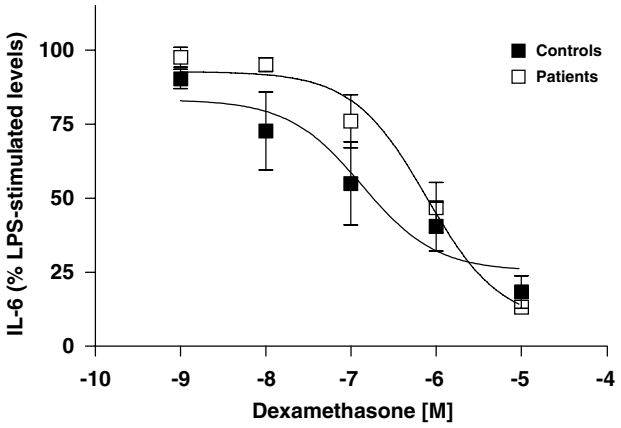


**Figure 1** Dexamethasone inhibition of LPS-stimulated IL-6 levels in diluted whole blood of healthy subjects (open squares, $n = 4$–9) and treatment-resistant depressed patients (closed squares, $n = 3$–8, in duplicate). Results are expressed as mean ± SEM of the % dexamethasone inhibition (LPS-stimulated IL-6 levels with glucocorticoid divided by LPS-stimulated IL-6 levels without glucocorticoids).

showing HPA axis changes by antidepressants (Glotzbach and Preskorn, 1982), and most importantly, are achieved in the brain of patients taking therapeutic doses of antidepressants. In fact, *in vivo* neuroimaging studies using spectroscopy, which are only possible with antidepressants containing fluorine atoms such as fluoxetine and fluvoxamine, have consistently described steady-state brain concentrations of these drugs in the micromolar range (Bolo *et al*, 2000). Moreover, brain concentrations of tricyclics in humans, largely derived from post-mortem studies after overdoses, have described brain-to-plasma concentration ratios ranging from 8-fold, at higher plasma concentrations, to 125-fold, at lower plasma concentrations (Bickel *et al*, 1967; Avella *et al*, 2004; Sunshine and Baeumler, 1963). Therefore, considering that the plasma concentrations of tricyclics in patients taking therapeutic doses range from 100 to 250 ng/ml (that is, approximately 0.3−0.8 μM for clomipramine), even a conservative estimate of a brain-to-plasma concentration of 10-fold would lead to micromolar concentrations of tricyclic antidepressants in the brain of patients. It is also of note that Dr Carroll's comment that only 10% of the drugs in animal brain is free could also apply to our *in vitro* assay, as there is an abundance of protein from plasma in the culture milieu. In any case, we have now run a toxicity assay incubating diluted whole blood cells for 24 h in the presence or absence of clomipramine 10 μM. Clomipramine does not change the number of monocytes ($0.41 \pm 0.02 \times 10^9$/l in vehicle *vs* $0.37 \pm 0.26 \times 10^9$/l in CMI 10 μM, $p = 0.69$, $n = 7$) or lymphocytes ($1.9 \pm 0.8 \times 10^9$/l in vehicle *vs* $1.7 \pm 0.08 \times 10^9$/l in CMI 10 μM, $p = 0.64$, $n = 7$). These data indicate that clomipramine is not toxic to cells in our assay. In further support to these data are our recent results in a human hippocampal stem cell line showing that in fact antidepressants, including clomipramine (1 μM), actually counteract glucocorticoid-induced inhibition of proliferation rather than being toxic for neurones (Anacker *et al*, personal communication).

In conclusion, we believe that the hypothesis of impaired GR function in patients with major depression is valid, and that our conclusions regarding the effect of clomipramine on GR function are indeed important for understanding the mechanism of action of antidepressants. We believe that our reply to Dr Carroll's queries is adequate and that this letter will be accepted for publication in *Neuropsychopharmacology*.

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