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SHORT COMMUNICATION

The chemical interaction between adrenochrome, three different classes of antipsychotic drugs and metabolites of the kynurenine pathway

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Abstract
Two kynurenine metabolites, 3-hydroxykynurenine and 3-hydroxyanthranilic acid, are known to inhibit melanin polymer formation in *in vitro* reactions catalyzed by tyrosinase. The present study expands that finding to include inhibition of chlorpromazine-stimulated melanin formation from the endogenous melanin precursor adrenochrome. Several kynurenine pathway metabolites tested had no measurable effect on the reaction: tryptophan, kynurenine, kynurenic acid, quinolinic acid and nicotinic acid. However, at a concentration of 0.5 mM in a pH 7.4 reaction mix, 3-hydroxykynurenine exerted ~72% inhibition on product formation and the same concentration of 3-hydroxyanthranilic acid caused complete inhibition. Two other classes of antipsychotic drugs were evaluated in this paradigm, represented by olanzapine and minocycline. Although the adrenochrome reaction of both drugs was strongly inhibited by 3-hydroxyanthranilic acid, 3-hydroxykynurenine inhibited product formation from only the minocycline reaction. The results are discussed in terms of the well-studied kynurenine pathway upregulation in psychotic disorders and how such upregulation may either influence the efficacy of antipsychotic drug treatment or relate to the mechanism of action of these drugs.

1. Introduction

Kynurenine pathway metabolites have been shown to be upregulated in individuals with psychotic disorders (Miller et al., 2008; Schwarcz et al., 2012), most of whom receive

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treatment with antipsychotic drugs subsequent to their diagnosis. The mechanism of action of these drugs has not yet been definitively determined (Richtand et al., 2007) and their efficacy varies from individual to individual (Remington and Kapur, 2010) in a manner that is not always related to specific blood level values (Buchanan et al., 2010; Zhang and Malhotra, 2011). Why this is so is not yet understood. The possible role of drug interactions with endogenous substrates other than dopaminergic and serotonergic receptors should be considered. For example, antipsychotic drugs exhibit a propensity to bind to melanin (Buszman et al., 2008) and to stimulate melanin formation *in vivo* (reviewed by Miller, 2013) and *in vitro* (Miller, 2013), leading some scientists to propose that at least few such psychoactive drugs may actually be consumed in the reaction to form subunits of the final polymeric product (Claffey et al., 2001). In such a scenario, neuromelanin formation could theoretically "draw down" the local brain concentration of antipsychotics in a manner that would not likely have an immediate effect on the drug blood levels.

Prior work by others had demonstrated that two kynurenine metabolites, 3-hyroxykynurenine and 3-hydroxyanthranilic acid, strongly inhibit the enzymatic formation of melanin polymer from melanin precursors (Soddu et al., 2004), raising the possibility that non-enzymatic melanin production might be similarly affected. The present study examines the interaction between kynurenine pathway metabolites and the chlorpromazine-stimulated (non-enzymatic) formation of polymeric melanin from the endogenous melanin precursor and catecholamine breakdown product, adrenochrome. The pH optima for chlorpromazine-stimulated product formation had previously been shown to lie close to 6.9 (Miller, 2013) and thus the preliminary screening experiments and dose response studies were carried out at this pH to optimize product formation attributable to chlorpromazine before extending the study to a more typical physiologic pH. As chlorpromazine is representative of first-generation antipsychotics, a representative of second-generation antipsychotics (olanzapine) and a novel antipsychotic (minocycline) were also tested in the experimental paradigm.

2. Experimental procedure

Adrenochrome and chlorpromazine-HCL were purchased from Toronto Research Chemicals (Toronto, Canada). Olanzapine and minocycline were purchased from Santa Cruz Biotechnology (Santa Cruz, *CA*). Tryptophan, kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid and nicotinic acid were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Concentrated stock solutions of the reagent chemicals were made in distilled/deionized water with the exception of olanzapine which was dissolved in ethyl acetate, and therefore, the minus-olanzapine control reactions contained the matching volume of ethyl acetate. Ethyl acetate itself had been previously shown to have no effect on the reaction (Miller, 2013). Reactions (1 ml final volume) between chlorpromazine and adrenochrome were carried out in 16×125 mm borosilicate glass tubes, unstirred, at room temperature and shaded from direct light. Controls employed the common reaction mix minus the experimental variable and were run in parallel with the experimental reactions. Comparisons between experiments in some cases involved different adrenochrome preparations, each with their appropriate controls, as noted in the figure captions. The reaction conditions were described and optimized for concentration, pH and temperature previously (Miller, 2013), and in brief, were derived from the original report by Galzigna (1972) who noted an effective *in-vitro* concentration of chlorpromazine and adrenochrome (500 μM) and from work by others who noted that pH values lower than 7.0 helped prevent adrenochrome decay in solution (Green et al., 1956). EDTA was included to complex any possible silver contamination from the adrenochrome production process as reviewed previously (Miller, 2013). Iron is known to enhance melanin formation (Bowness and Mortone, 1953) and was therefore included at a physiologic level, ~10 μM (Kemna et al., 2005). The common reaction buffer contained (final concentration): 1x phosphate-buffered-saline (PBS); 75 μM EDTA; and 1.6 μg/ml Fe2O3 (10 μM equivalent). The final concentration of the reaction substrates were 500 μM each (adrenochrome, antipsychotic drugs and kynurenine pathway metabolites). Stock solutions of adrenochrome, chlorpromazine-HCl and minocycline were stored at 4 °C until use (within 2 weeks). Stock solutions of olanzapine in ethyl acetate were stored at −20 °C until use (within 2 weeks). Stock solutions of tryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid and nicotinic acid were made up in distilled/deionized water and frozen until use (up to several months).

The reactions containing chlorpromazine or minocycline were allowed to progress for seven days, while those containing olanzapine were allowed to progress for 10 days before measurements were made, based on prior work (Miller, 2013). For all reactions, after vortexing vigorously, aliquots were taken at the reaction end point and diluted 10-fold. The optical density (absorbance at 660 nm) was measured using a Varian Cary Bio-300 (Agilent Inc, Wilmington, DE), scanning spectrophotometer (the absorption results for 660 nm are reported here), completed within 4 min of vortexing. The decline in measured values from particulate downward drift was <2% in 10 min of standing in the cuvette. Reported values in the text and figures represent the mean ± SD; significant changes were calculated using a student's t-test.

3. Results

As reported previously (Miller, 2013), chlorpromazine reacted with adrenochrome to form an insoluble product that could be measured by the increase in absorbance at 660 nm (A_{660}, Fig. 1, upper plots) and directly visualized in the melanin-like precipitate formed after spinning the reaction in a microcentrifuge tube (see Miller, 2013). Under the conditions employed, and lacking the milieu of cofactors likely present *in vivo*, the control (adrenochrome alone) did not autopolymerize to form melanin over the course of the seven day incubation (Fig. 1, lower left plots).

Of the kynurenine precursors and metabolites tested (Fig. 1), 3-hydroxykynurenine and 3-hydroxyanthranilic acid had a significantly negative impact on the product formation from the chlorpromazine-adrenochrome reaction (Fig. 1, middle panels, upper plots), whereas the other additions (tryptophan, kynurenine, kynurenic acid, quinolinic acid, niacin) had no significant effect. The effect of varying the concentration of the two inhibitors was then

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