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Opposite effects of tolcapone on amphetamine-disrupted startle gating in low vs. high COMT-expressing rat strains

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Abstract

Background—Differential sensitivity to the prepulse inhibition (PPI)-disruptive effects of dopamine agonists in Sprague–Dawley (SD) vs. Long Evans (LE) rats is heritable, reflects differential activation of DA signaling, and is associated with differences in the brain expression of specific genes, including those of the catecholamine catabolic enzyme, catechol-O-methyltransferase (COMT). In humans, both basal and drug-modified PPI differs significantly between individuals with polymorphisms conferring low- vs. high-activity of COMT. We used the COMT inhibitor, tolcapone, to assess the role of COMT activity in regulating the differential effects of the dopamine releaser, amphetamine (AMPH), on PPI in SD and LE rats.

Methods—Acoustic startle and PPI were assessed in SD and LE male rats after pretreatment with tolcapone (vehicle vs. 30 mg/kg ip) and treatment with AMPH(vehicle vs. 4.5 mg/kg sc), using 10–120 ms prepulse intervals.

Results—After tolcapone, AMPH significantly potentiated PPI in LE rats, and significantly disrupted PPI in SD rats. These patterns could not be explained by drug effects on pulse alone startle magnitude.

Discussion—The impact of COMT inhibition on AMPH-modified PPI was categorically different in strains exhibiting low vs. high levels of forebrain *Comt* expression, consistent with reports in humans that tolcapone has opposite effects on PPI among individuals with polymorphisms conferring low vs. high COMT activity. The present model provides a basis for understanding the mechanisms by which the effects of COMT inhibition on sensorimotor gating – and potentially, related neurocognitive and clinical functions – under hyperdopaminergic states are dependent on an individual's basal levels of COMT activity.

Keywords

Amphetamine; Dopamine; Catechol-O-methyltransferase; Prepulse inhibition; Schizophrenia; Strain — tolcapone

1. Introduction

Genes conveying low vs. high levels of catechol-O-methyltransferase (COMT) activity are associated with high vs. low levels of neurocognitive performance in healthy as well as schizophrenia subjects, and with differential sensitivity to neurocognitive and behavioral effects of drugs, including the COMT-inhibitor, tolcapone (Giakoumaki et al., 2008; Roussos et al., 2008, 2009). Individuals carrying the Val/Val genotype of the Val158Met *COMT* polymorphism (rs4680), conferring high COMT activity, exhibit increases in working memory and sensorimotor gating – as measured by prepulse inhibition of startle (PPI) – after a single dose of tolcapone (Giakoumaki et al., 2008); in contrast, individuals carrying the Met/Met genotype of rs4680 tend to exhibit reduced PPI after tolcapone. Similar findings were reported with working memory by a separate group (Farrell et al., 2012), and were also detected with a second *COMT* polymorphism (rs4818) (Roussos et al., 2009). Because schizophrenia patients exhibit deficits in working memory and PPI, these studies support the premise that pharmacologic manipulations of COMT activity might have procognitive effects in biomarker-identified schizophrenia patients (Apud and Weinberger, 2007; Bitsios and Roussos, 2011).

We previously reported different effects of dopamine (DA) agonists on PPI in rat strains distinguished by lower (Sprague Dawley (SD)) or higher (Long Evans (LE)) levels of *Comt* expression in the nucleus accumbens, medial prefrontal cortex and ventral hippocampus (Shilling et al., 2008; Swerdlow et al., 2012). In rats, DA agonists, such as the DA releaser, amphetamine (AMPH), generally reduce PPI with longer (60–120 ms) prepulse intervals, and increase PPI with shorter (30 ms) prepulse intervals (e.g. Talledo et al., 2009). Here, we investigated whether AMPH effects on PPI were sensitive to COMT inhibition with tolcapone, and whether – like in humans – tolcapone's effects were dependent on basal levels of COMT activity.

2. Methods

Sixteen SD and 16 LE male rats (229–250 g) (Harlan, Livermore, California) were housed and handled as in past reports (e.g. Shilling et al., 2008). Studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the UCSD Animal Subjects Committee (protocol #S01221).

Tolcapone (VWR) was dissolved in a saline/0.2% Tween 80 vehicle, then sonicated intermittently until administered intraperitoneally (i.p.; vehicle or 30 mg/kg) 80 min prior to AMPH. AMPH (Sigma, 4.5 mg/kg) or vehicle (saline) were administered subcutaneously (sc) 10 min prior to testing. The dose, route of administration and pretreatment time for tolcapone were based on published reports (Tunbridge et al. 2004; Laatikainen et al., 2012). These parameters for AMPH produce two critical strain-specific “phenotypes”: PPI-enhancing effects of AMPH at short (30 ms) prepulse intervals, and PPI-disruptive effects of AMPH at long (60–120 ms) prepulse intervals (Talledo et al., 2009; Swerdlow et al., 2012); full dose–response effects of AMPH on long-interval PPI in SD and LE rats are published (Swerdlow et al., 2003, 2007).

Startle chambers (SR-LAB; SDI, San Diego, CA) were in a sound-attenuated room (60 dB(A) ambient noise). A brief startle session was used to form balanced drug groups according to average %PPI. Testing began 7 days later. Rats were pretreated with either saline/tween vehicle or 30 mg/kg tolcapone followed 80 min by either saline or 4.5 mg/kg AMPH, and placed in the startle chambers 10 min later. After 7 days, this was repeated, with AMPH dose reversed and dose order balanced across pretreatment dose- and strain-groups. Startle tests were 16 min long and consisted of 5 min of 70 dB(A) background noise

followed by 6 trial types: PULSE (118 dB(A) 40 ms noise burst) alone, or preceded 10, 20, 30, 60 or 120 ms earlier by a prepulse (5 ms noise burst, 15 dB over background). Inter-trial intervals were variable and averaged 15 s; “hidden” in each inter trial interval was a 100 ms period during which chamber activity was measured without stimulus presentation (“NOSTIM” trials).

Data were analyzed as in past reports (e.g. Shilling et al., 2008; Talledo et al., 2009). ANOVAs assessed startle magnitude, NOSTIM values, and PPI, using strain and tolcapone dose as between-subject factors, and AMPH dose as a within-subject factor, along with trial block (for measures of startle magnitude and NOSTIM activity) and prepulse interval (for measures of PPI). Post-hoc comparisons were made using a Fisher's PLSD test. Alpha was 0.05.

3. Results

In all analyses of startle magnitude (Fig. 1A) and NOSTIM activity (data not shown), there were no significant main or interaction effects involving tolcapone. ANOVA of startle magnitude revealed that only the interaction of strain $\times$ AMPH dose reached statistical significance ($F = 5.79$, df 1,28, $p < 0.025$). This interaction reflected the reported tendency for AMPH to enhance startle magnitude in LE rats (here reaching trend levels, $p < 0.065$) but not SD rats (e.g. Talledo et al., 2009). Similarly, for NOSTIM amplitude, among all main, 2- and 3-way interaction effects, only the interactions of strain $\times$ AMPH dose ($F = 5.55$, df 1,28, $p < 0.03$) and strain $\times$ AMPH dose $\times$ trial block ($F = 7.18$, df 2,56, $p < 0.002$) reached statistical significance. These interactions reflected the reported tendency for AMPH to increase NOSTIM activity in SD rats (here reaching trend levels ($p < 0.075$)) but not LE rats (e.g. Talledo et al., 2009).

Tolcapone had opposite effects on AMPH-modified PPI in LE vs. SD rats (Fig. 1B). ANOVA of PPI revealed no significant main effects (all F 's < 1), but significant 2-way interactions of AMPH dose $\times$ strain ($F = 8.86$, df 1,28, $p < 0.007$) and AMPH dose $\times$ trial ($F = 24.52$, df 4,112, $p < 0.0001$), and a significant 3-way interaction of AMPH dose $\times$ strain $\times$ tolcapone dose ($F = 10.62$, df 1,28, $p < 0.003$), and no other informative 2-, 3- or 4-way interactions. The 3-way interaction of AMPH dose $\times$ strain $\times$ tolcapone dose reflected the fact that after tolcapone pretreatment, AMPH significantly increased PPI in LE rats ($p < 0.01$), and significantly decreased PPI in SD rats ($p < 0.04$). Based on previous findings of strain differences in AMPH PPI effects at short vs. long prepulse intervals, we confirmed this identical pattern with 30 ms PPI (Fig. 1C, ovals: AMPH dose $\times$ strain $\times$ tolcapone dose interaction: $F = 7.96$, df 1,28, $p < 0.009$; among tolcapone-pretreated rats, AMPH increased PPI in LE rats ($p < 0.008$) and decreased PPI in SD rats ($p < 0.05$)). At long prepulse intervals (60–120 ms; Fig. 1C, rectangles), tolcapone blocked the PPI-reducing effect of AMPH in LE rats, but not in SD rats: among tolcapone-treated rats, ANOVA revealed a significant interaction of strain $\times$ AMPH dose ($F = 7.90$, df 1,14, $p < 0.015$), reflecting the significant PPI-reducing effects of AMPH in SD ($p < 0.005$) but not LE rats (ns).

4. Discussion

This study demonstrated that after tolcapone, AMPH has opposite effects on PPI in rats with high (LE) vs. low (SD) levels of brain *Comt* expression (Shilling et al., 2008; Swerdlow et al., 2012). After tolcapone, AMPH significantly increased PPI in LE rats, and significantly decreased PPI in SD rats. These opposite effects reflected two temporally distinct processes. In LE rats, tolcapone potentiated AMPH's PPI-enhancing effects at short intervals, and blocked AMPH's PPI-reducing effects at long intervals. In SD rats, tolcapone blocked AMPH's PPI-enhancing effects at short intervals, and potentiated AMPH's PPI-reducing

effects at long intervals. While the paradigm is not fully homologous, these findings parallel those demonstrated in humans, in which tolcapone produced opposite effects on PPI and neurocognitive performance in healthy subjects carrying polymorphisms conferring low vs. high levels of COMT activity (Giakoumaki et al., 2008; Roussos et al., 2009; Farrell et al., 2012).

Surely, lower levels of brain regional *Comt* expression in SD vs. LE rats cannot be viewed as physiologically comparable to lower levels of COMT enzymatic conferred by the Met allele in rs4680. We reported lower basal levels of forebrain DA turnover in SD vs. LE rats (Swerdlow et al., 2005), suggesting at least that brain regional *Comt* expression differences may have functional consequences. Furthermore, our animal model uses amphetamine to provoke excessive DA release that “uncovers” the physiological impact of baseline circuit differences in these strains, while in humans, phenotypic differences are evident in untreated “Val/Val” vs. “Met/Met” individuals at “baseline” (e.g. Giakoumaki et al., 2008; Roussos et al., 2008). Thus, this model based on differential regional brain expression levels in rats is not viewed as isomorphic to a condition based on a genetic polymorphism in humans.

Systemic drug effects on behavior are not easily interpreted at a neural level, but other information about these rat strains might help clarify possible mechanisms for these strain-dependent effects of tolcapone on PPI. In addition to lower basal levels of forebrain DA turnover in SD vs. LE rats (Swerdlow et al., 2005), we reported differences in DA signaling and its impact on striato-pallidal GABAergic outputs in these strains (Bitsios et al., 2006; Qu et al. 2009; Swerdlow et al. 2011). Furthermore, we (Swerdlow et al., 2006) and others (Bubser and Koch, 1994) reported regional brain differences in the DAergic regulation of PPI, particularly between the nucleus accumbens (NAC: long interval PPI reduced by D2 stimulation) and medial prefrontal cortex (mPFC: long interval PPI reduced by D1 blockade), suggesting that post-tolcapone increases in PPI in LE rats vs. decreases in PPI in SD rats may reflect predominant roles for the mPFC vs. NAC, respectively. Clearly, we do not yet know the causal relationship between differences in regional *Comt* expression and those in forebrain PPI regulatory circuitry between SD and LE rats. Presumably, systemically-administered tolcapone alters PPI and amphetamine sensitivity indirectly, via changes within descending projections from the forebrain to primary startle circuits in the pons. We are currently grappling with methodological complexities of localized tolcapone delivery to forebrain regions known to differ in *comt* expression, so that we can test specific mechanistic hypotheses.

Given the heterogeneity of schizophrenia, there is a clear need to identify and understand biomarkers that predict sensitivity to particular procognitive drug effects. Tolcapone appears to have biomarker-predictable effects on neurocognition in healthy individuals (Giakoumaki et al., 2008; Roussos et al., 2008, 2009; Farrell et al., 2012), and studies in progress are examining such effects in schizophrenia cohorts. Beyond the intuitive parsimony that a COMT-inhibitor should have differential effects on individuals with low vs. high basal COMT activity, there must be specific neural circuit processes underlying such genotype-specific drug effects. Conceptual models have invoked “inverted-U” functions for DA activity and neurocognition as a way to understand the relationship between *COMT* genotype and tolcapone effects on cognition and behavior (Farrell et al., 2012). The present findings suggest that it will be possible to identify more specific neural mechanisms underlying COMT-dependent effects of tolcapone on sensorimotor gating and neurocognition in rodents.

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Fig. 1.

Tolcapone and AMPH effects on startle and PPI in Sprague Dawley (SD) and Long Evans (LE) rats. A. Drug and strain effects on startle magnitude: Data revealed only a previously-reported tendency for AMPH to enhance startle magnitude in LE rats but not SD rats. B. Drug and strain effects on PPI, collapsed over the 10–120 ms prepulse intervals: Tolcapone had opposite effects on AMPH-modified PPI in LE vs. SD rats: after tolcapone pretreatment, AMPH significantly increased PPI in LE rats ($p < 0.01$) (see “up” arrow; **), and significantly decreased PPI in SD rats ($p < 0.04$) (see “down” arrow; *). Collapsed across this time course, data appear to suggest that in rats pretreated with tolcapone vehicle, AMPH had no effect on PPI; however, when examined across the 10–120 ms interval (Fig. 1C),

robust biphasic effects of AMPH are evident in vehicle-pretreated rats of both strains. C. Drug and strain effects on PPI across prepulse intervals: At short (30 ms; see ovals) intervals, tolcapone enhanced AMPH-induced increases in PPI in LE rats (left oval), and blocked AMPH-induced increases in PPI in SD rats (right oval). At long intervals (120 ms; see rectangles), tolcapone blocked the PPI-reducing effect of AMPH in LE rats (left rectangle), but not in SD rats (right rectangle). In tolcapone-treated rats, ANOVA revealed significant PPI-reducing effects of AMPH in SD ($p < 0.005$) but not LE rats (ns).