Studies on Prostaglandin-Endoperoxide Synthase 1: Lower Levels in Schizophrenia and After Treatment with Antipsychotic Drugs in Conjunction with Aspirin

Brian Dean, Andrew Gibbons, Andrea Gogos, Madhara Udawela, Elizabeth Thomas, Elizabeth Scarr

The Florey Institute for Neuroscience and Mental Health, Parkville, Victoria, Australia (Drs Dean, Gibbons, Gogos, Udawela, and Scarr); Centre for Mental Health, Swinburne University of Technology, Hawthorn, Australia (Dr Dean); Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Victoria, Australia (Dr Scarr); The Scripps Research Institute, La Jolla, California (Dr Thomas).

Correspondence: Brian Dean, HNDApp.Biol., LI Biol., MSc, PhD, The Molecular Psychiatry Laboratory, The Florey Institute for Neuroscience and Mental Health, 30 Royal Parade, Parkville, Victoria, 3052, Australia (brian.dean@florey.edu.au).

Abstract

Background: Antipsychotic drugs plus aspirin (acetylsalicylic acid), which targets prostaglandin-endoperoxide synthase 1 (PTGS1: COX1), improved therapeutic outcomes when treating schizophrenia. Our microarray data showed higher levels of PTGS1 mRNA in the dorsolateral prefrontal cortex from subjects with schizophrenia of long duration of illness, suggesting aspirin plus antipsychotic drugs could have therapeutic effects by lowering PTGS1 expression in the cortex of subjects with the disorder.

Methods: We used Western blotting to measure levels of PTSG1 protein in human postmortem CNS, rat and mouse cortex, and cells in culture.

Results: Compared with controls, PTGS1 levels were 41% lower in the dorsolateral prefrontal cortex (P < .01), but not the anterior cingulate or frontal pole, or subjects with schizophrenia. Levels of PTGS1 were not changed in the dorsolateral prefrontal cortex in mood disorders or in the cortex of rats treated with antipsychotic drugs. There was a strong trend (P = .05) to lower cortical PTGS1 10 months after mice were treated postnatally with polyinosinic–polycytidylic acid sodium salt (Poly I:C), consistent with cortical PTGS1 being lower in adult mice after exposure to an immune activator postnatally. In CCF-STTG1 cells, a human-derived astrocytic cell line, aspirin caused a dose-dependent decrease in PTGS1 that was decreased further with the addition of risperidone.

Conclusions: Our data suggest low levels of dorsolateral prefrontal cortex PTGS1 could be associated with the pathophysiology of schizophrenia, and improved therapeutic outcome from treating schizophrenia with antipsychotic drugs augmented with aspirin may be because such treatment lowers cortical PTGS1.

Keywords: Schizophrenia, aspirin, COX1, PTSG1, postmortem, cortex
**Significance Statement**

We showed that levels of PTGS1 messenger RNA were higher in the DLPFC from subjects with schizophrenia of long duration of illness. This was significant, because PTGS1 is targeted by aspirin, which increases the ability of antipsychotic drugs to lessen the symptoms of schizophrenia. Here, we report levels of PTGS1 protein is lower in the DLPFC, but not anterior cingulate or frontal pole, from subjects with schizophrenia and that PTGS1 is not altered in subjects with mood disorders or rats treated with antipsychotic drugs. Levels of PTGS1 were lower in human-derived astrocytes treated with aspirin, an effect augmented by the edition of the antipsychotic drug, risperidone. We propose lower levels of PTGS1 protein in the DLPFC are part of the pathophysiology of schizophrenia, and treatment with aspirin combined with an antipsychotic drug gives improved therapeutic benefits by lowering PTGS1 in subjects with schizophrenia.

**Introduction**

There is a significant body of evidence that suggests inflammation is contributing to the pathophysiology of schizophrenia (Na et al., 2014). This hypothesis is supported by studies that have shown changes in levels of mRNA for genes involved in modulating inflammatory-related pathways in postmortem CNS tissue from subjects with schizophrenia (Saetre et al., 2007; Narayan et al., 2008; Schmitt et al., 2011; Hwang et al., 2013). Significantly, our study of CNS gene expression showed that, compared with controls, levels of prostaglandin-endoperoxide synthase 1 (PTGS1: alias cyclooxygenase 1 [COX1]) mRNA were significantly higher in the dorsolateral prefrontal cortex from subjects with schizophrenia who were older than 40 years (average age = 64±7.8 years) (Narayan et al., 2008; Tang et al., 2012b).

This finding is of significance with regards to therapeutic interventions, as it has been shown that acetylsalicylic acid (aspirin), which targets PTGS1, lessens the severity of some of the symptoms of schizophrenia when given as an adjunctive treatment with antipsychotic drugs compared with what is achieved giving antipsychotic drugs alone (Laan et al., 2010; Weiser et al., 2012). One of these clinical studies noted that acetylsalicylic acid adjunctive treatment was more effective in subjects with high levels of blood interferon γ:interleukin 4 ratios, a measure of increasing inflammatory status (Laan et al., 2010), data suggesting that treatment with acetylsalicylic acid was more effective in subjects with schizophrenia who were experiencing an inflammatory episode. The second study (Weiser et al., 2012) showed that acetylsalicylic acid, but not minocycline which is a tetracycline antibiotic with anti-inflammatory effects that does not act on PTGS1, improved symptom severity when given with antipsychotic drugs to subjects with schizophrenia. These data suggest that targeting PTGS1 could be an important mechanism of action for a drug that, when given with an antipsychotic drug, improves therapeutic outcomes.

Acetylsalicylic acid acts by targeting PTGS1 and PTGS2 (Vane and Botting, 2003), which both convert arachidonic acid into bioactive prostanooids. Until recently it was thought that PTGS1 was responsible for physiological production of prostanooids, whereas PTGS2 was involved in proinflammatory pathways being inducible at sites of inflammation (Aid and Bosetti, 2011). However, PTGS1 has now been shown to be present in microglia, where it is suggested to have a major role in modulating CNS inflammatory processes (Choi et al., 2009). Hence, elevated levels of PTGS1 expression in the CNS could be indicative of generalized inflammatory processes driven by activated microglia in subjects with schizophrenia (van Berckel et al., 2008; Doorduin et al., 2009). By contrast, PTGS2 is predominantly localized to neurons and may therefore be more relevant to inflammatory processes that affect only those cells.

Our studies showing higher levels of PTGS1 in mRNA in the dorsolateral prefrontal cortex from subjects with schizophrenia (Narayan et al., 2008; Tang et al., 2012b) differed from findings at the level of protein that suggested PTGS1 was not different in prefrontal cortex, temporal cortex, or occipital cortex from subjects with the disorder (Maida et al., 2006). Thus, putting together our data and data on PTGS1 protein, it could be that there are cortical region-specific changes in PTGS1 expression in the CNS from subjects with schizophrenia or changes in PTGS1 expression are not translating into changes in levels of PTGS1 protein. To address this issue, we decided to determine if PTGS1 protein was changed in the same cortical regions where there were higher levels of mRNA in subjects with schizophrenia. We also decided to measure levels of PTGS1 in the frontal pole and anterior cingulate cortex from subjects with schizophrenia to determine if changes in PTGS1 could be present throughout the cortex. To address the hypothesis that levels of PTGS1 are altered in the CNS after an inflammatory episode, we measured levels of PTGS1 in the cortex of neonatal mice treated with polyinosinic-poly-cytidylic acid (Poly I:C), which activates inflammatory pathways (Ibi et al., 2009). We also measured PTGS1 in the cortex of mice that had been treated neonatally with phencyclidine hydrochloride (PCP), because this model, rather than models that deliver the drug to the rat foetus in utero (Jones et al., 2011), is not thought to act through inflammatory or immune pathways (Anastasio and Johnson, 2008). Significantly, both these animal models produce a behavioral phenotype that allows the study of specific aspects of schizophrenia. We also sought to determine if treatment with an antipsychotic drug alone could affect levels of PTGS1 in rat cortex. Finally, we addressed the hypothesis that treatment with acetylsalicylic acid with antipsychotic drugs affects levels of PTGS1 by measuring levels of the protein in CCF-STTG1 cells, an eternalized cell line derived from a human astrocytoma (Vik-Mo et al., 2009), which we have established stably express PTGS1, treated with vehicle, acetylsalicylic acid (aspirin), or acetylsalicylic acid and risperidone.

**Methods**

**Materials**

Poly I:C and PCP were obtained from Bio-Scientific Pty Ltd. Haloperidol, risperidone, and all general chemicals were obtained from Sigma-Aldrich Pty Ltd. CCF-STTG1 cells were obtained from American Type Culture Collection. RPMI 1960 media, fetal calf serum (FCS), and antibiotic-antimycotic (AB-AM) were obtained from ThermoFisher Scientific. Anti-PTGS1
antibody was obtained from Abcam (cat no.: AB109025). The goat anti-rabbit IgG: horseradish peroxidase complex was obtained from DAKO. All electrophoresis grade chemicals were from BioRad.

Ethical Approval

The Ethics Committee of the Victoria Institute for Forensic Medicine gave approval to collect human CNS post-mortem, and all animal experiments were conducted with the approval of the Florey Institute for Neuroscience and Mental Health Animal Ethics Committee.

Studies in Human CNS

CNS tissue was collected from people who had a likely history of a psychiatric disorder and subjects who displayed no obvious symptoms of psychiatric disorders and who had not died by suicide (controls). At the time of death, no case had measurable levels of acetylsalicylic acid in their blood. Tissue was collected from donors after either a witnessed death or having been seen alive within 5 hours of being found dead. All cadavers were refrigerated soon after discovery, which is advantageous as this would aid in slowing the effects of autolysis (Ferrer et al., 2007). To further optimally preserve CNS tissue, the left hemisphere from each donor was processed in a standardized manner that ensured tissue was frozen to -80°C within 30 minutes of autopsy (Dean et al., 1999). Just prior to freezing, a sample of CNS tissue was collected from each donor, and CNS pH was measured as described previously (Kingsbury et al., 1995), because CNS pH is a good indicator as to the quality of tissue preservation (Stan et al., 2006).

For each case, relevant data from clinical histories and interviews with treating clinicians and relatives were obtained using a standardized instrument, the Diagnostic Instrument for Brain Studies (Hill et al., 1996). On completion of this review, in cases other than those where it was agreed the donor had no history of psychiatric illness (controls), a diagnosis was made by consensus by 2 psychiatrists and a psychologist according to DSM-IV criteria (Roberts et al., 1998). In addition, the postmortem interval (PMI) was calculated as either the time from witnessed death to autopsy or the time mid-way between a subject being last seen and being found dead until autopsy. Duration of illness was calculated as the time from first presentation with psychiatric symptoms to death. The final recorded doses of different classes of psychotropic drugs were also recorded and standardized to a dose of a specific drug in class (Foster, 1989).

For this study, tissue from the dorsolateral prefrontal cortex (Brodman’s [BA] area 46) was obtained from 20 subjects with schizophrenia, 20 subjects with major depressive disorders, 17 subjects with bipolar disorders, and 20 controls. Tissue was also obtained from BA 10 and BA 24 from the subjects with schizophrenia and the controls. Efforts were made to match age and sex across diagnoses. Different regions of the cortex were dissected guided by cytotectonic markers (Garey, 1994); hence, BA 10 was taken as the most rostral portion of the superior frontal gyrus and middle frontal gyrus, bounded ventrally by the superior rostral sulcus; BA 24 as anterior cingulate gyrus around the genu of the corpus callosum; and BA 46 as the lateral surface of the frontal pole, including approximately the middle third of the middle frontal gyrus.

Studies in Rodent CNS

Studies in Rats

In determining the potential effects of antipsychotic drug treatment on PTGS1, we decided to use an archetypal, first-generation, antipsychotic drug, because this was the class of drugs used to treat the cases of subjects with schizophrenia used in this study prior to death. Hence, 6-week-old male Sprague Dawley rats were exposed to a 12-h-day/-night schedule with free access to food. The rats (n = 10) received 0.1 mg/kg/d haloperidol in vehicle or vehicle alone (ethanol 0.1% v/v) in their drinking water for 28 days. This dose of drug was given on the principle that it would give a dopamine-D2 receptor occupancy in a rat equivalent to what is regarded as being optimal when treating subjects with schizophrenia (Kapur et al., 2003) but adjusted for delivery of haloperidol in drinking water rather than minipumps (0.25 mg/kg/d) or by s.c. injection (0.04–0.08 mg/kg s.c./d) over 1 month rather than 7 days. The dose of haloperidol used in this study was slightly higher than that used for i.p. injection, as we have shown this gives blood levels of the drug in rats equivalent to what would be expected in humans (Scarr and Dean, 2012). The drug doses were adjusted twice weekly based on the average daily water consumption and increasing weight of each rat. After the treatment, the rats were left untreated for 48 hours and then sacrificed by decapitation and the brains removed and rapidly frozen and stored at -80°C.

Studies in Mice

Neonate C57black6j mice were housed with their dams in a 12-h-light/-dark cycle with free access to food and water. Two groups of 20 neonate mice received either daily i.p. injections of pyrogen-free saline or pyrogen-free saline containing 10 mg/kg Poly I:C between postnatal days (PD) 2 to 6. All mice were immediately returned to their dam after each injection. Two groups of 20 mice were injected i.p. on PD 7, 9, and 11 with either pyrogen-free saline or pyrogen-free saline containing 10 mg/kg PCP and on each occasion immediately returned to their dam. All mice were weaned at PD 21, after which the sexes were housed separately. From each treatment group, 10 mice were killed by cervical dislocation at 1 month of age, which is at a prepubescent phase, whilst the remaining 10 mice were killed postpuberty at 10 months of age (Dutta and Sengupta, 2016). Notably, just before being killed, the Poly I:C mice and the PCP mice were tested with the saccharin preference test. At 1 month old, neither set of treated mice showed altered saccharin preference but at 10 months the Poly I:C-treated, but not the PCP-treated mice, showed reduced saccharin preference, which is viewed as a behavior analogous to the anhedonia in schizophrenia (Jones et al., 2011). Immediately following cervical dislocation, the CNS was harvested and the frontal cortex was dissected and rapidly frozen in an isopentane bath on dry ice and stored at -80°C until needed. Whole brain from one of the control groups dams was used to prepare the internal control used in Western blotting (see below).

Studies with CCF-STTG1 Cells

For the study of the impact of acetylsalicylic acid + risperidone on PTGS1 levels, we chose to use CCF-STTG1 cells, because they express human PTGS1 stably in culture. In these experiments, we used risperidone, because that was the antipsychotic drug with the least complex neuropsychopharmacology (Goldstein, 2000) of the drugs used in the study where acetylsalicylic acid was used as an adjunctive treatment and caused an improvement in symptom severity (Laan et al., 2010). Thus, CCF-STTG1
Cells were cultured to 70% confluency in RPMI 1960 media containing 10% heat inactivated FCS and 1x AB-AM at 37°C with 5% CO₂. Cells, at a density of 5 x 10⁴ cells/well, were then subcultured (P9) into 6-well cell culture plates and grown for 10 days in RPMI 1960 media containing 5% FCS and 1x AB-AM. The media was then removed, cells washed with 1x phosphate buffered saline and replaced with RPMI 1960 media containing 1x AB-AM, no FCS, and either 0, 0.1, 0.5, or 1.0 mM acetylsalicylic acid in the absence or presence of risperidone (1 or 10 µM). The pH of all media preparations was measured and, where necessary, adjusted to pH 7.4 prior to commencing the study. Five replicate wells were prepared for each combination of drugs. The cells were harvested after 24 hours and washed thrice in 1x phosphate buffered saline. Protein homogenates were prepared by sonicating the cells in 10 mM Tris, pH 7.4, 1% SDS, and 1 mM Na₃VO₄ and stored at -80°C until needed. Protein homogenate prepared from CCF-STTG1 cells cultured in RPMI 1960 media containing 10% FCS and 1x AB-AM and harvested at 70% confluency was used as an internal control for Western blotting (see below).

Western Blotting

Human and rat (frontal cortex) tissue was homogenized into 10 mM Tris containing 1% sodium dodecyl sulphate and 1 mM sodium orthovanadate (pH 7.4) (5 x vol/tissue wet weight) using 20 strokes using a Potter-Elvehjem PTFE pestle and glass tube. The protein concentration in each homogenate was then measured using the modified BioRad Detergent Compatible assay (Lowry method). Cortical homogenates, containing 20 µg of protein, were then separated in duplicate on a 7.5 % polyacrylamide resolving gel. The separated proteins were transferred onto nitrocellulose membranes (overnight constant at 40 mA in Towbin transfer buffer). Each membrane was stained with 0.2% Ponceau S in 3% TCA to confirm effective transfer of proteins.

After destaining, membranes were blocked with 5% non-fat milk powder (NFMP) at room temperature (R/T) for 1 hour and then incubated with anti-PTGS1 antibody (ABCAM, cat no.: ab109025 Anti-COX1 / Cyclooxygenase 1 rabbit monoclonal antibody), clone no.: EPR5866 at a dilution of 1/200 in Tris-Tween-Buffer-Saline (TTBS) with 5% NFMP overnight at 4°C. Membranes were then washed thrice for 10 minutes at R/T in TTBS and then exposed to a 1/2000 dilution of goat anti-rabbit IgG: horseradish peroxidase in TTBS with 5% NFMP for 1 hour at R/T. Membranes were washed thrice for 10 minutes at R/T in TTBS and then incubated with Pierce ECL solution for 5 minutes at R/T. Excess ECL solution was then removed from the nitrocellulose, an image of chemiluminescence captured using a Kodak 440 CF imaging system. The intensity of the PTGS1 immunogenic band at ~70 kDa was then measured on each nitrocellulose gel.

In many studies, Western blot results have been expressed normalized to a loading control (Aldridge et al., 2008), the notion of the loading control being that the level of the chosen protein will not vary as a proportion of total protein between tissues or between individuals. However, it is becoming increasingly clear that loading controls and/or reference genes or proteins are not tenable for studies using human CNS tissue where levels of individual proteins vary as a proportion of total protein between donors (Eaton et al., 2013). Thus, the best way to control for protein loading, as we have done in this study, is to carefully measure the protein concentration of each sample immediately prior to it being loading onto the gel. This ensures that the same protein concentration is loaded for each case (Eaton et al., 2013). Moreover, as described previously (Dean et al., 2006), to control for inter-blot variation, an internal control (IC) of cortical homogenate was prepared from a single case that was not used in the current study. This homogenate was run under the exact conditions to be used for the cases of interest in every lane on 2 gels and the intensity of the immunogenic band of interest measured in each lane (30 measurements). These measurements were used to establish both intra- and interblot variation for the measurement of PTGS1. This sample was subsequently run (in duplicate) on every gel, and each gel was exposed so that the sum intensity of the IC fell within the range established by calculating the mean ±2 SD of the initial 30 measurements. The intensity of PTGS1 from all cases were the standardized by expressing them as a ratio of IC. For studies in rats and mice, a similarly prepared IC from rat and mice cortex was used.

Statistics

Demographic, CNS collection, and psychotropic drug data were analyzed using the D’Agostino and Pearson omnibus normality test, and nonnormally distributed data were subsequently analyzed using nonparametric statistics. Student t tests or 1-way ANOVA were used to compare age, CNS pH, PMI, brain weight, and duration of illness. By contrast, experimental data expressed as ratios are better analyzed with nonparametric statistics (Siegel, 1957; Allison et al., 1995; Curran-Everett, 2013; Dean et al., 2016). PTGS1 levels in BA 10 and 24 from subjects with schizophrenia and controls were compared using the Mann-Whitney U test. Suicide and gender ratios across diagnoses were compared using the Fisher exact test, and comparisons of levels of PTGS1 across gender and between suicide completers and death by other causes was by Mann-Whitney U test. Comparisons of experimental and with demographic, CNS collection, and pharmacological data were compared using the Spearman’s rank correlation coefficient. Where there were strong relationships between experimental and nonexperimental data, comparisons across diagnoses were made using nonexperimental data as covariates.

The data comparing levels of PTGS1 in the cortex of rats receiving vehicle or treated with haloperidol were compared using the Mann-Whitney U test. Levels of PTGS1 at 1 month and 10 months after treatments with Poly I:C or PCP were compared using the Mann-Whitney U test. Data from cell culture data were analyzed using Kruskal-Wallace test using Dunn’s test as a posthoc analysis to compare the effects of risperidone and acetylsalicylic acid at different doses with the vehicle alone. Statistical analyses were completed using GraphPad Prism version 6.00 for Windows, GraphPad Software.

Results

Case History Reviews

There were no significant differences in age or PMI across diagnoses (Table 1; supplementary Table 1). There was a significant variation in gender ratio between controls and subjects with major depressive disorders as well as subjects with schizophrenia and subjects with major depressive disorders. CNS pH was significantly higher in subjects with major depressive disorders compared with controls, subjects with schizophrenia, and subjects with bipolar disorders. There was a significant variation in CNS weight across diagnostic cohorts, but no individual diagnosis differed from any other diagnoses. Rates of suicide completion were higher in subjects with major depressive disorders compared with subjects with schizophrenia and subjects with
bipolar disorders. Levels of final recorded antipsychotic drug, lifetime exposure to antipsychotic drug, final recorded dose of antidepressant drug, or final recorded dose of mood stabilizers did not differ significantly between subjects with schizophrenia, major depressive disorders or bipolar disorders.

Antibody Validation

There is a growing concern about the specificity of antibodies used in various immuno-detection techniques (Manimala et al., 2007; Jositsch et al., 2009); thus, we determined the immunogenic profile of the anti-human PTGS1 antibody used in this study using human cortex and a homogenate of human embryonic kidney 293T cells that had either been, or not been, transfected with the human PTGS1 gene. Using Western blotting, we showed the presence of a single immunogenic band of molecular weight 70 kDa in 2 samples of human cortex (Figure 1A). An immunogenic band of the same molecular weight was present at high levels in the homogenate from 293T cells transfected with the human PTGS1 gene. When comparing levels of cortical PTGS1 across diagnoses, there were no significant relationships between levels of PTGS1 and age, PMI, CNS weight, duration of illness, final recorded antipsychotic drug, final recorded dose of antidepressant drug, or final recorded dose of mood stabilizers when comparing levels of cortical PTGS1 across diagnoses. There were no significant relationships between levels of PTGS1 and age, PMI, CNS weight, duration of illness, final recorded antipsychotic drug, final recorded dose of antidepressant drug, and final recorded dose of mood stabilizers (Table 2), suggesting these were not potential confounds.

Studies in Rodent Cortex

Compared with vehicle treatment, levels of PTGS1 did not differ in the cortex of rats treated with 0.1 mg/kg/d haloperidol for 28 days (Figure 2A). Similarly, compared with vehicle treatment, levels of PTGS1 did not differ in the cortex of mice 1 month after treatment with 10 mg/kg Poly I-C (Figure 2B), but there was a strong trend to decreased levels of PTGS1 in the cortex of mice 10 months after such treatment (P = .05). Indeed, a power analysis of these data using G*Power Version 3.1.9.2 showed that an addition of 1 animal per treatment group at 10 months would be needed to reach significance P < .05. Compared with vehicle treatment, levels of PTGS1 did not differ in the cortex of mice 1 or 10 months after treatment with 10 mg/kg PCP.

Studies in CCF-STTG1 Cells

Compared with vehicle treatment, levels of PTGS1 did not differ in CCF-STTG1 cells after exposure to 1 or 10 μM risperidone for 24 hours (Figure 3A). By contrast, acetylsalicylic acid caused a dose-dependent decrease in levels of PTGS1 in CCF-STTG1 cells after treatment for 24 hours, which was significantly lower in BA 46 (P < .01), but not BA 10 (P = .37) or BA 24 (P = .08), from subjects with schizophrenia (Figure 1B). A power analysis of the data from BA 24 using G*Power version 3.1.9.2 showed that 92 individuals in each diagnostic group would be needed to reach significance (P < .05). Levels of PTGS1 were measured only in BA 46 from subjects with major depressive disorders and bipolar disorders and did not differ when compared with controls (Figure 1B).

Levels of PTGS1 did not differ with gender in BA 10 (median ± IQR: F = 1.31 (0.88–1.55) vs M = 1.34 (1.02–1.54), P = .74), BA 24 (median ± IQR: F = 1.12 (0.96–1.51) vs M = 1.09 (0.76–1.84), P = .96), or BA 46 (median ± IQR: F = 1.67 (1.03–2.47) vs M = 1.78 (1.15–2.44), P = .73). For those with a psychiatric diagnosis, levels of PTGS1 did not differ between suicide completers and subjects who died from other causes in BA 10 (median ± IQR: suicide completers = 1.18 (0.97–1.53) vs other = 1.16 (0.88–1.56), P = .73), BA 24 ((median ± IQR): suicide completers = 0.99 (0.67–1.16) vs other = 1.01 (0.65–1.60), P = .61), or BA 46 (median ± IQR: suicide completers = 1.72 (1.28–2.39) vs other = 1.35 (1.02–2.00), P = .27). These data suggest that whilst gender ratio and suicide varied with diagnoses (Table 1), they were not potential confounds when comparing levels of cortical PTGS1 across diagnoses.

Table 1. A summary (for numeric: mean ± SEM) of data collected during the case history reviews of subjects with no history of psychiatric illness (controls) as well as for subjects with schizophrenia, major depressive disorders and bipolar disorders.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age (yr)</th>
<th>Sex (M / F)</th>
<th>pH</th>
<th>PMI (hr)</th>
<th>CNS Weight (Kg)</th>
<th>DI (yr)</th>
<th>Suicide (y / n)</th>
<th>FRADD</th>
<th>LEAP</th>
<th>FRAnDD</th>
<th>FRMSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROLS</td>
<td>49 ± 4.7</td>
<td>16 / 4</td>
<td>6.28 ± 0.04</td>
<td>41 ± 3.5</td>
<td>1.43 ± 0.34</td>
<td>0 / 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCHIZOPHRENIA</td>
<td>48 ± 4.7</td>
<td>16 / 4</td>
<td>6.32 ± 0.05</td>
<td>43 ± 3.1</td>
<td>1.43 ± 0.35</td>
<td>21 ± 4.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAJOR DEPRESSIVE DISORDERS</td>
<td>56 ± 3.6</td>
<td>9 / 11</td>
<td>6.54 ± 0.04</td>
<td>40 ± 3.6</td>
<td>1.30 ± 0.43</td>
<td>16 ± 2.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BIPOLAR DISORDERS</td>
<td>56 ± 3.4</td>
<td>9 / 8</td>
<td>6.29 ± 0.04</td>
<td>40 ± 4.0</td>
<td>1.23 ± 0.12</td>
<td>17 ± 3.0</td>
<td></td>
<td></td>
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</tbody>
</table>

Abbreviations: BD = bipolar disorders, Cont = Controls, DI = duration of illness, F = female, FRADD = final recorded antipsychotic drug does converted to mg chlorpromazine equivalents per day, FRAnDD = final recorded antidepressant drug dose converted to mg fluoxetine equivalents per day, FRMSD = final recorded mood stabiliser dose converted to mg lithium equivalents per day, hr = hours, Kg = kilogram, LEAP = lifetime exposure to antipsychotic drugs converted to as chlorpromazine equivalents per year x 10⁻³, M = male, MDD = major depressive disorder, n = no, Sz = schizophrenia, y = yes, yr = year.
Acetylsalicylic acid decreased levels of PTGS1 in CCF-STTG1 cells in the presence of both 1 μM and 10 μM risperidone with differences between drug treatment and vehicle reaching significance at 0.5 M acetylsalicylic acid.

Discussion

We have shown that levels of PTGS1 are lower in the dorsolateral prefrontal cortex, but not frontal pole or anterior cingulate cortex, from subjects with schizophrenia and that PTGS1 levels are not altered in the dorsolateral prefrontal cortex from subjects with major depressive disorders or bipolar disorders. Our previous data showed higher levels of PTGS1 mRNA in the dorsolateral prefrontal cortex from subjects with schizophrenia of long duration (Narayan et al., 2008; Tang et al., 2012b). By contrast, unlike for levels of mRNA, we do not find that differences between levels of PTGS1 protein in BA 46 from subjects with schizophrenia, compared with controls, vary in significance with duration of illness. Thus, it is possible that lower levels of PTGS1 in BA 46 are present at or close to the onset of schizophrenia and that the higher levels of expression of PTGS1 in BA 46 from subjects with schizophrenia of long duration (Narayan et al., 2008) represent a slow and unsuccessful response to rectify low protein levels. Alternatively, changes in gene promoter methylation are known to occur with age (Jung and Pfeifer, 2015) and duration of illness also correlates with age. Therefore, the increased expression of PTGS1 mRNA in schizophrenia may be associated with differential changes in gene promoter methylation with age in BA 46 from subjects with schizophrenia and controls (Tang et al., 2012a). However, PTGS1 protein should more closely reflect functional changes in the CNS, and therefore we would

Table 2. The relationships between levels of prostaglandin-endoperoxide synthase 1 in (A) human BA 10, BA 24 and BA 46 and with (B) case history data in those three cortical regions.

<table>
<thead>
<tr>
<th>Age</th>
<th>pH</th>
<th>PMI</th>
<th>CNS Weight</th>
<th>DI</th>
<th>FRADD</th>
<th>FRAntiD</th>
<th>FRMSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA 24</td>
<td>0.19</td>
<td>0.25</td>
<td>0.13</td>
<td>0.42</td>
<td>-0.19</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>BA 46</td>
<td>-0.09</td>
<td>0.46</td>
<td>-0.01</td>
<td>0.92</td>
<td>0.06</td>
<td>0.58</td>
<td>-0.08</td>
</tr>
<tr>
<td>BA 10</td>
<td>0.00</td>
<td>0.99</td>
<td>0.00</td>
<td>0.99</td>
<td>0.03</td>
<td>0.85</td>
<td>-0.16</td>
</tr>
</tbody>
</table>

Abbreviations: BA = Brodmann’s area, DI = duration of illness, FRADD = final recorded antipsychotic drug dose expressed as chlorpromazine equivalents per day, FRAntiD = final recorded antidepressant drug dose expressed as fluoxetine equivalents per day, FRMSD = final recorded dose of mood stabiliser drug dose expressed as lithium equivalents per day, PMI = postmortem interval

different from vehicle at 1.0 M acetylsalicylic acid (Figure 3B). Acetylsalicylic acid decreased levels of PTGS1 in CCF-STTG1 cells in the presence of both 1 μM and 10 μM risperidone with differences between drug treatment and vehicle reaching significance at 0.5 M acetylsalicylic acid.

Figure 1. Measuring levels of PTGS1 in human and rodent cortex. (A) A Western blot showing levels of a protein that displayed immunogenic sites that for an anti-prostaglandin synthase 1 (PTGS1) antibody (ABCAM Anti-COX1 / Cyclooxygenase 1 rabbit monoclonal antibody, clone no.: EPR5866) in human, rat, and mouse cortices (20 μg protein) as well as 293T cells that were either transfected or not transfected with the human prostaglandin-endoperoxide synthase 1 (PTGS1) gene. To provide an impression of relative levels of expression 20 μg protein from undiluted 293T or CCF-STTG1 cell lysate (set 3) or cell lysate diluted 1:10 (set 2) or 1:20 (set 1) prior to loading onto the polyacrylamide gel are shown. (B) A typical Western blot showing levels of a 70-kDa protein that displayed immunogenic sites for an anti-PTGS1 antibody in homogenates from Brodmann’s areas 10, 24 and 46 from subjects with schizophrenia and age / sex nonpsychotic controls. (B) Levels (median ± interquartile range) of PTGS1 protein in cortical homogenates containing 20 μg total protein from BA 10, 24, and 46 from subjects with schizophrenia and age / sex-matched controls, as well as BA 46 from subjects with major depressive disorders and bipolar disorders.
argue that PTGS1 in BA 46 is either involved in or affected by the pathophysiology of the disorder.

Notably, our data suggest that low levels of PTGS1 may be localized to the dorsolateral prefrontal cortex in subjects with schizophrenia. This argument is in line with findings from an earlier study, which, using immunohistochemistry (Maida et al., 2006) or Western blotting (Rao et al., 2013), failed to show any change in levels of PTGS1 protein in BA 8, 18, 21, or 22, or hippocampus or PTGS1 mRNA in BA 10 from subjects with schizophrenia (Maida et al., 2006). In addition, we report no change in levels of PTGS1 in the dorsolateral prefrontal cortex from subjects with major depressive disorders and bipolar disorders, which adds to earlier findings that the protein is not changed in prefrontal cortex, temporal cortex, or occipital cortex from subjects with mood disorders (Maida et al., 2006). There is a report of lower levels of PTGS1 protein and mRNA in the frontal cortex from subjects with bipolar disorder (Kim et al., 2012). However, the region of the frontal cortex used was not defined and may not be one of the cortical regions used in this study.

Current data would suggest that changes in PTGS1 levels do not occur globally in the CNS from subjects with schizophrenia,
and therefore the changes in PTGS1 may make a unique contribution to the changes in functioning of the dorsolateral prefrontal cortex that have long been associated with schizophrenia. Significantly, PTGS1 has recently been proposed to form part of a protein-protein interactome that is predicted to be affected because of genetic changes that have been shown to increase the risk for schizophrenia (Ganapathiraju et al., 2016). Moreover, many of the proteins in this interactome, including PTGS1, were predicted to be promising drug targets for drugs to treat schizophrenia. Hence, our data would encourage further study of the PTGS1 containing interactome, as this may reveal potential sites other than PTGS1 that may have therapeutic benefits in people with schizophrenia (Laan et al., 2010; Weiser et al., 2012); such drugs may not have the unwanted side effects associated with the long term use of aspirin (Ridker et al., 2005).

We have begun to explore mechanisms that have the potential to lower levels of PTGS1 in the CNS of subjects with schizophrenia. We have shown that treating rats or cells in culture with the anti-psychotic drugs, haloperidol, and risperidone, respectively, does not change levels of PTGS1. Our rat treatment data agree with an earlier study that reported treating rats with haloperidol at 0.15 mg/kg/d for 14 days did not change levels of Pts1 expression (Wong et al., 2003). By contrast with studies in rats using haloperidol, a study treating rats with 6 mg/kg/d olanzapine for 21 days reported no change in PTGS1 protein, but, compared with vehicle, found higher levels of Ptg1 mRNA in the frontal cortex (Cheon et al., 2011). These outcomes were the same as a study that reported that, compared with vehicle, treating rats with clozapine at 10 mg/kg/d for 30 days resulted in higher levels of Ptg1 mRNA without affecting levels of PTGS1 protein (Kim et al., 2012). The same study reported that treatment with clozapine also reduced the activity of PTGS1. Importantly, whilst data on clozapine and olanzapine raise the interesting possibility that some of the therapeutic effects achieved with these drugs may involve modulating Pts1 mRNA levels and/or activity, none of the data suggest the lower levels of PTGS1 in BA 46 are simply due to treatment with antipsychotic drug.

Here we report that levels of cortical PTGS1 were very close to being lower in 10-month-old postpubescent mice (Dutta and Sengupta, 2016), but not in 1-month-old prepubescent mice who had been injected with Poly I:C soon after birth. Such a change in cortical PTGS1 was not observed in mice 1 or 10 months after PCP injections close to birth. These data are significant, because only in the 10-month-old Poly I:C-treated mice were we able to demonstrate a decreased sucrose preference; this change in behavior is thought to be analogous to anhedonia in schizophrenia (Jones et al., 2011). Hence, our data raise the possibility that Poly I:C treatment during the postnatal period causes immune activation to bring about a behavioral phenotype in adult mice that models some aspects of the pathophysiology of schizophrenia (Ibi et al., 2009). In addition, our data also suggest that some changes in response to neonatal immune activation do not become apparent in the CNS until after puberty and that one of these changes may result in lower levels of PTGS1. We postulate that lower levels of PTGS1 in BA 46 from subjects with schizophrenia could be a response to a process involving immune activation some considerable time prior to death and would support the notion of immunity and inflammation being involved in the pathophysiology of the disorder.

We wished to understand the effects of treating with acetylsalicylic acid and risperidone, as was done in subjects with schizophrenia (Laan et al., 2010; Weiser et al., 2012) on levels of PTGS1. We have now shown a dose-dependent decrease in levels of PTGS1 after exposing CCF-STTG1 cells to acetylsalicylic acid, which was further enhanced in the presence of risperidone. Our data therefore suggest there may be a synergistic interaction between antipsychotic drugs, such as risperidone, with acetylsalicylic acid to increase its ability to lower levels of PTGS1. Our data from a human-derived astrocytic-like cell line differs from that from human monocyte cell lines, which, after exposure to acetylsalicylic acid, did not change levels of PTGS1 mRNA or protein (Barrios-Rodiles et al., 1996). These data suggest that acetylsalicylic acid may have cell-specific effects on PTGS1 levels and that these effects may differ in the CNS and the periphery. Moreover, our data suggest interactions between drugs such as acetylsalicylic acid and antipsychotic drugs may be particularly beneficial in the treatment of schizophrenia. This argument is supported by the finding that the nonselective PTGS inhibitor, naproxen, antagonized haloperidol-induced catalepsy in mice (Naidu and Kulkarni, 2002). Notably, this effect was not observed with the selective PTGS2 inhibitor, rofecoxib, suggesting the interaction between naproxen and haloperidol involved PTGS1. Importantly, it is still not known if the therapeutic response to treatment with acetylsalicylic acid and risperidone is due to a CNS-wide reduction in levels of PTGS1 in subjects with schizophrenia or by lowering further levels of PTGS1 in BA 46 from subjects with the disorder. It would seem unlikely that treatment with acetylsalicylic acid and risperidone would have regionally selective effects, and therefore at present it would seem most likely that the extra benefits obtained by using acetylsalicylic acid as an adjunctive treatment with risperidone involves a CNS or cortical-wide reduction in PTGS1. Hence, preclinical and clinical data would suggest exploring the potential interactions between drugs such as acetylsalicylic acid and antipsychotic drugs, as this could reveal potential therapeutic benefits associated with co-administration of such drugs in subjects with schizophrenia (Laan et al., 2010; Weiser et al., 2012).

As with all studies of proteins involved in dynamic processes, our data on levels of PTGS1 could be affected by factors such as the use of anti-inflammator medications, diet, or comorbid inflammatory illness. In addition, although no case used in this study had recordable levels of acetylsalicylic acid in their blood at death, it is likely many individuals had taken acetylsalicylic acid during their lifetime. However, although the long-term use of acetylsalicylic acid is used to treat a number of conditions, there seems to be no data on how quickly any changes in PTGS1 levels revert to normal after cessation of treatment. Unique to all postmortem studies, it is possible a particular cause of death may affect levels of cortical PTGS1. In addition, there would likely be a high level of substance abuse comorbidity in the subjects with psychiatric disorders. For example, there are high levels of nicotine, cannabis, and cocaine use in subjects with schizophrenia (Winklbaur et al., 2006). It is therefore significant that nicotine has been shown to increase, not decrease, the expression of PTGS1 (Schör et al., 1998). By contrast, tetrahydrocannabinol has been shown not to affect levels of PTGS1 (Chen et al., 2013). We could find no evidence to suggest cocaine affects levels of PTGS1. Thus, available data suggest that the changes in levels of PTGS1 in BA 46 from subjects with schizophrenia are not due to substance abuse.

In summary, our data suggest there are lower levels of PTGS1 in the dorsolateral prefrontal cortex of subjects with schizophrenia and that treatment with acetylsalicylic acid acts to lower levels of that protein, at least in a human-derived astrocytic-like cell line, an outcome enhanced by the presence of an antipsychotic drug. The synergistic action of antipsychotic drugs and acetylsalicylic acid on levels of PTGS1 provides a potential mechanism of action by which treating subjects with
schizophrenia with these 2 classes of drugs could improve therapeu tic outcomes compared with treatment with antipsychotic drugs alone (Laan et al., 2010). However, an observational cohort study using data from the Danish nationwide registries suggests there are multiple outcomes linked to the use of antipsychotic drugs and nonsteroidal antiinflammatory drugs, such as aspirin, in treating schizophrenia (Köhler et al., 2016). One suggested disadvantage of using acetylsalicylic acid or diclofenac was an increased risk of relapse and hospitalization. These data would need to be taken into account when considering the use of PTGS1/PTGS2 targeting drugs as an adjunctive treatment for schizophrenia. If our data from cell culture models the effects of treatment with antipsychotic drugs and acetylsalicylic acid on PTGS1 levels in the human dorsolateral prefrontal cortex, one interpretation of our data is that such treatment is helping to further amplify endogenous changes in cortical PTGS1 that are trying to mitigate pathophysiological process occurring in schizophrenia. It could also be argued that the therapeutic benefit from augmenting antipsychotic drug treatment with acetyl salicylic acid is because this decreases levels of PTGS1 in regions of the CNS other than BA 46. This study cannot resolve this issue but does suggest that, given the important role for PTGS1 in CNS inflammation (Choi et al., 2009), lower levels of PTGS1 in the CNS from subjects with schizophrenia should be protective against neuroinflammation (Fillman et al., 2013).

Supplementary Material
Supplementary data are available at International Journal of Neuropsychopharmacology online.

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Statement of Interest
None.

References
increased expression of immune/inflammation-related genes in the hippocampus of individuals with schizophrenia. Transl Psychiatry 3:e231.


Sae et al. | 225


