Inhibition of stress-induced hepatic tryptophan 2,3-dioxygenase exhibits antidepressant activity in an animal model of depressive behaviour

Sinead M. Gibney1,2, Eimear M. Fagan2, Ann-Marie Waldron1, Jordan O’Byrne1, Thomas J. Connor2 and Andrew Harkin1

1 Neuropsychopharmacology Research Group, School of Pharmacy and Pharmaceutical Sciences & Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland
2 Neuroimmunology Research Group, Department of Physiology, School of Medicine & Trinity College Institute of Neuroscience, Trinity College, Dublin 2, Ireland

Abstract

The role of hepatic tryptophan 2,3 dioxygenase (TDO) was assessed in the provocation of stress-induced depression-related behaviour in the rat. TDO drives tryptophan metabolism via the kynurenine pathway (KP) and leads to the production of neuroactive metabolites including kynurenine. A single 2 h period of restraint stress in adult male Sprague–Dawley rats provoked an increase in circulating concentrations of the glucocorticoid corticosterone and induction of hepatic TDO expression and activity. Repeated exposure to stress (10 d of 2 h restraint each day) provoked an increase in immobility in the forced swimming test (FST) indicative of depression-related behaviour. Immobility was accompanied by an increase in the circulating corticosterone concentrations, expression and activity of hepatic TDO and increase in the expression of TDO in the cerebral cortex. Increased TDO activity was associated with raised circulating kynurenine concentrations and a reduction in circulating tryptophan concentrations indicative of KP activation. Co-treatment with the TDO inhibitor allopurinol (20 mg/kg, i.p.), attenuated the chronic stress-related increase in immobility in the FST and the accompanying increase in circulating kynurenine concentrations. These findings indicate that stress-induced corticosterone and consequent activation of hepatic TDO, tryptophan metabolism and production of kynurenine provoke a depression-related behavioural phenotype. Inhibition of stress-related hepatic TDO activity promotes antidepressant activity. TDO may therefore represent a promising target for the treatment of depression associated with stress-related disorders in which there is evidence for KP activation.

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Introduction

Tryptophan is an essential amino acid that acts as a precursor for the biosynthesis of the neurotransmitter serotonin (5-HT). The majority of available tryptophan is, however, metabolised via the kynurenine pathway (KP). The rate-limiting enzymes in this pathway are indoleamine 2,3 dioxygenase (IDO) and tryptophan 2,3 dioxygenase (TDO). IDO is ubiquitous throughout the body and is known to be activated by inflammatory cytokines (Carlin et al., 1989; Fujigaki et al., 2006; Zunszain et al., 2012). TDO is primarily found in the liver and is activated by stress-induced corticosteroids (Green and Curzon, 1975; Green et al., 1975a, b, c; Young, 1981; Danesch et al., 1987; Nakamura et al., 1987; Ren and Correia, 2000). As well as potentially reducing the availability of tryptophan for 5-HT synthesis, activation of the KP leads to the formation of kynurenine metabolites, which can act on the glutamatergic system in the central nervous system (CNS) (Muller and Schwarz, 2007; Chen and Guillemin, 2009).

Activation of IDO in response to immune challenge has been reported to affect tryptophan availability and the occurrence of depressive symptoms (Capuron et al., 2002; Schiepers et al., 2005). In addition, preclinical investigations indicate that IDO activation mediates the depressive-like behavioural effects of a systemic immune challenge (O’Connor et al., 2009). Despite these findings, a body of pre-clinical data argues against the notion that IDO activation in response to an immune challenge results in a reduction of 5-HT synthesis. For instance a number of studies have consistently reported increased tryptophan availability in the CNS following administration of bacterial lipopolysaccharide (LPS) or other inflammagens (Dunn et al., 1989; Moroni et al., 1991;...
Dunn, 1992a, b). Moreover, there is no evidence of central 5-HT depletion following administration of LPS or inflammatory cytokines to animals; in fact, an increase in 5-HT release and metabolism coupled with increased activity of tryptophan hydroxylase has been observed in rats following a systemic inflammatory challenge with LPS (Nolan et al., 2000; Dunn et al., 2005). Also recent clinical studies argue against IDO-mediated depletion of 5-HT synthesis in depressed patients (Wichers et al., 2005; Myint et al., 2007) and it has been suggested that the action of kynurenine metabolites on the glutamatergic system may be involved in producing depressive symptoms (Muller and Schwarz, 2007). In any case reports to date support a link between KP activation and the production of depression-related symptoms in patients and animal models.

Curzon and Green (1969) demonstrated that hepatic TDO activity increases significantly following exposure of rats to periods of restraint stress representing an alternative mechanism to immune stimulation whereby the KP may be activated. Systemic administration of the stress hormone hydrocortisone produced similar effects. Corresponding changes in central 5-HT concentrations were noted in which restraint stress or corticosterone administration provoked a reduction in 5-HT. Adrenalectomy attenuated the stress-related increase in hepatic TDO, indicating that increased TDO activity is triggered by activation of the hypothalamus–pituitary–adrenal (HPA) axis (Green and Curzon, 1968; Curzon and Green, 1969). Activation of TDO may also influence the production of KP metabolites where administration of hydrocortisone has been reported to increase circulating concentrations of kynurenine in rats (Young, 1981). Despite the evidence to indicate that stress and corticosteroids increase hepatic TDO activity, there have been no investigations to date which assess the role of TDO in stress-induced depression-related behaviour.

The forced swimming test (FST) is one of the most commonly used screening tests for antidepressants in rodents (Porsolt et al., 1978; Borsini and Meli, 1988; Borsini, 1995; Cryan et al., 2005). More recently, the FST has been adopted to assess an endophenotype associated with animal models of depression in which immobility in the FST is increased by prior exposure to stressors (Armario et al., 1991; Veena et al., 2009; Kim et al., 2012) and by genetic factors which may increase vulnerability to stress and depression (Cryan and Mombereau, 2004; Jacobson and Cryan, 2007; Solomon et al., 2012). In addition, repeated daily sessions of restraint stress have been shown to provoke an increase in immobility time in the FST in rats (Gigliucci et al., 2013).

The objective of the present investigation was to determine if the KP is activated by induction of hepatic TDO in response to acute and chronic stressor exposure in rats and to elucidate a role for TDO and activation of the KP in the stress-induced increase in immobility in the FST.

Methods

Animals

Male Sprague–Dawley rats (250–350 g) were obtained from Harlan, UK. Rats were maintained on a 12 h light: 12 h dark cycle (lights on at 08:00 h) in a temperature-controlled room (22±2 °C) and food and water were available ad libitum. The experimental protocols were in compliance with the European Communities Council directive (86/609/EEC).

Restraint stress

Rats were immobilised in well-ventilated perspex cylindrical restrainers (35-cm length×7-cm internal diameter; Harvard Apparatus, UK). For the acute stress protocol animals were restrained for 2 h and euthanised immediately after, 2 and 24 h following cessation of stress (n=6 per group). For chronic stress the animals were restrained for 2 h for 8 consecutive days. On day 9 the animals were not restrained but were exposed to the FST (see below for details). On day 10 animals were restrained for 2 h and euthanised immediately after, 2 and 24 h following cessation of stress (control, n=12; stress, n=6 per group).

Forced swimming test (FST)

The FST was performed as previously described (Gigliucci et al., 2010). On day 8 of chronic stress animals were placed in a cylindrical container (40 cm×18 cm) filled with water (temp 22–25 °C) and subjected to a 15 min pre-swim 2 h after exposure to restraint on that day. The water depth was such that the animals had to swim or float without their hind limbs touching the bottom of the container. On day 9, animals were exposed to the FST for a second time for 5 min and time spent immobile during this period was recorded. Immobility was determined as the absence of escape-orientated behaviour (swimming, diving, rearing or climbing), except movements that were necessary to stay afloat and keep the head above water.

Allopurinol treatment

Allopurinol is a purine analogue that is commonly used for the treatment of gout as it is an inhibitor of the enzyme xanthine oxidase, which in turn inhibits the formation of uric acid (Pacher et al., 2006). It is thought to mediate its effects on the inhibition of TDO by preventing the conjugation of TDO with its co-factor heme, thus maintaining it in its inactive form (Badawy and Evans, 1973; Welch and Badawy, 1980). Allopurinol (20 mg/kg) has been repeatedly shown to inhibit TDO activity induced by both stress and exogenous corticosterone administration (Becking and Johnson, 1967; Curzon and Green, 1969; Julian and Chytil, 1970; Green et al., 1976).
Animals were exposed to the chronic restraint stress protocol as outlined above. Each day, immediately before insertion into the restrainers animals received an intra-peritoneal injection of either vehicle (saline; n=8) or allopurinol (20 mg/kg; Sigma-Aldrich, Ireland; n=8). Animals were subjected to the 15 min pre-swim on day 8 of the stress protocol, 2 h after exposure to restraint stress on that day. On day 9, animals received an injection of either vehicle or allopurinol 5 and 1 h prior to the second 5 min test exposure to the FST. The drug administration protocol followed is standard for testing pharmacological agents in the rat FST (Porsolt et al., 1977; Harkin et al., 1999; Connor et al., 2000; Durkin et al., 2008). Immobility was recorded over the 5 min duration of the test. Animals were then dried and returned to the home cage. Animals were euthanised 24 h post the final stress exposure; this time point was chosen as it was the time post stress at which increased immobility and circulating kynurenine concentrations were apparent in previous experiments. TDO activity measurements were not carried out as TDO expression and activity return to control levels by 24 h following cessation of stress.

**Real-time PCR**

Ribonucleic acid (RNA) was extracted from brain tissue using the NucleoSpin® RNA II total RNA isolation kit (Macherey-Nagel, Germany). Genomic deoxyribonucleic acid (DNA) contamination was removed by the addition of DNase to the samples. RNA was reverse transcribed into cDNA using a High-Capacity cDNA Archive Kit (Applied Biosystems, Germany). Real-time PCR was performed using an ABI Prism 7300 instrument (Applied Biosystems) as previously described (Connor et al., 2008). Taqman Gene Expression Assays (Applied Biosystems) containing forward and reverse primers and a Taqman® Universal PCR Master Mix and samples were run in duplicate. The cycling conditions consisted of 90 °C for 10 min and 40 cycles of 90 °C for 15 s followed by 60 °C for 1 min. β-actin was used as an endogenous control to normalise gene expression data. Relative gene expression was calculated using the ΔΔCT method with Applied BioSystems RQ software (Applied BioSystems, UK).

**Analysis of tryptophan and kynurenine concentrations**

Tryptophan and kynurenine were measured in serum samples using HPLC coupled to UV/Fluorescence detection as previously described (Gibney et al., 2013). The mobile phase contained 50 mm glacial acetic acid, 100 mm zinc acetate (Sigma, Ireland) and 3% acetonitrile dissolved in double-distilled NANOpure water HPLC grade H2O (Sigma, Ireland). The pH was adjusted to 4.9, using 5 M NaOH. Serum was diluted 1:1 in mobile phase containing 7% perchloric acid spiked with 50 ng/20 μl of N-methyl 5-HT (Sigma, Ireland) as internal standard. Samples were centrifuged at 20 000 rpm for 20 min and the supernatants were placed into new eppendorf tubes, using a syringe fitted with a 0.45 μm filter (Phenomenex, UK). 20 μl of the filtered supernatant was injected using a Waters autosampler and a Reverse Phase analytical column (Kinetex™ Core Shell Technology column with specific area of 100 mm×4.6 mm and particle size of 2.6 μ, Phenomenex, UK) was used for separation of metabolites. A PDA-UV detector (Shimadzu SPD-M10A VP), calibrated to integrate at 230 and 250 nm, as well as a fluorescent detector (Shimadzu RF-20A XS prominence fluorescence detector), set to excitation wavelength 254 nm; emission wavelength 404 nm, were used to detect the metabolites. Chromatographs were generated by CLASS-VP software (Shimadzu, UK). Results are expressed as ng of analyte per ml of serum.

**Determination of corticosterone concentrations**

Corticosterone concentration in serum was measured using a commercially available corticosterone enzyme immunoassay kit (Immunodiagnostic Systems, UK). The assay was performed according to the manufacturer’s instructions, and absorbance read at 450 nm using a microplate reader. Absorbance was recalculated as a concentration (ng/ml) using a standard curve derived using GraphPad Prism Software Version 4.00 (GraphPad Software, USA).

**Tryptophan 2, 3-dioxygenase activity assay**

The enzyme assay was modified from a method previously described (Badawy and Evans, 1975; Stowell and Morland, 1983; Sarkar et al., 2007). Liver tissue was homogenised in 10 mM phosphate buffered saline, pH 7.0 (10% w/v), sonicated for 2 min and then centrifuged @ 13 000 rpm for 10 min. The supernatant was removed and haematin (2 μM final concentration) and tryptophan (30 μM final concentration) were added. The solution was incubated in a shaking water bath for 60 min and the reaction stopped by the addition of trichloroacetic acid (0.09 M final concentration). The solution was then heated at 65 °C for 15 min to hydrolyse N-formylkynurenine to kynurenine. The solution was centrifuged again at 13 000 rpm for 5 min. Equal quantities of supernatant and Ehrlich’s reagent (in glacial acetic acid) were added to a 96-well micro-titer plate and absorbance measured at 480 nm. A standard curve of defined concentrations of L-Kynurenine (0–400 μM; Sigma, Ireland) was used to determine kynurenine concentrations in the supernatants (Braun et al., 2005). Protein concentration in the supernatants was quantified by a Bio-Rad Protein assay with absorbance measured at 595 nm. Bovine serum albumin was used for the standard curve. TDO activity in the supernatants is reported as nmols of...
kynurenine produced per milligram of protein per minute of the total assay time (60 min).

**Statistical analysis of data**

All values are expressed as mean±S.E.M. Data were analysed using a one-way (or two-way) analysis of variance (ANOVA) followed by a Newman–Keuls post-hoc test or an unpaired student-t-test (GB Stat). A value of $p<0.05$ was considered to be statistically significant.

**Results**

**Acute restraint stress leads to raised circulating corticosterone concentrations and an increase in hepatic TDO expression and activity**

Raised circulating concentrations of corticosterone were evident immediately after stress exposure ($p<0.001$), which returned to control levels 2 h following stress when compared to non-stressed controls (Fig. 1a; $n=6$ per group). A corresponding increase in hepatic TDO expression was observed in animals subjected to acute stress immediately following stress exposure ($p<0.001$). This expression remained significantly elevated for 2 h post stress ($p<0.001$), returning to control levels 24 h later when compared to non-stressed controls (Fig. 1b; $n=6$ per group). In addition hepatic TDO activity was increased 0 and 2 h post stress ($p<0.05$), returning to control activity levels 24 h later when compared to non-stressed controls (Fig. 1c; $n=6$ per group).

**Increased immobility time in the FST following chronic restraint stress and associated changes in corticosterone, TDO expression and activity**

The duration of immobility in the FST was increased in stressed animals compared to non-stressed controls ($p<0.001$, Fig. 2; control $n=12$; stress $n=24$). Raised circulating concentrations of corticosterone were evident immediately after cessation of the repeated stressor regime ($p<0.001$), which returned to control levels 2 h later when compared to non-stressed controls (Fig. 3a; control $n=6$ per group). An increase in hepatic TDO expression (Fig. 3b) and activity (Fig. 3c) in animals subjected to chronic stress was observed immediately and 2 h following cessation of stress ($p<0.001$; $p<0.05$ respectively) when compared to non-stressed controls (control $n=12$, stress $n=6$ per group). As with TDO expression and activity following acute stress exposure, there was a return to control levels 24 h following cessation of final stress exposure in the chronic stress regime. When TDO mRNA expression was measured in the frontal cortex no change was detected in response to acute stress when compared to non-stressed controls at any time point analysed (data not shown). In response to chronic stress however an increase in TDO expression was found immediately after and 2 h post stress when compared to non-stressed controls ($p<0.05$) returning to control levels 24 h later (Fig. 3d; control $n=12$, stress $n=6$ per group). Although changes in TDO expression and activity were evident following stress, no changes compared to non-stressed controls ($p<0.05$) returning to control levels 24 h later (Fig. 3d; control $n=12$, stress $n=6$ per group).
Inhibition of TDO promotes an antidepressant response

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**Increased TDO activation post chronic stress is associated with reduced circulating tryptophan and increased circulating kynurenine concentrations**

Although acute stress induced an increase in the expression and activity of hepatic TDO, no stress-induced changes in circulating concentrations of tryptophan or kynurenine were apparent (data not shown). However, the increase in hepatic TDO expression and activity in response to chronic stress was associated with a decrease in circulating tryptophan 2 h post stress (Fig. 4a; control n=12, stress n=6 per group) and an increase in circulating kynurenine 24 h post stress (Fig. 4b) when compared to non-stressed controls (p<0.05). An increase in the kynurenine/tryptophan ratio (Fig. 4c) 24 h post stress when compared to non-stressed controls (p<0.05) is indicative of increased systemic metabolism of tryptophan to kynurenine.

**Allopurinol blocks stress-related increases in immobility in the FST, reduced body weight gain and circulating kynurenine concentrations**

When non-stressed controls were treated with either saline or allopurinol, no difference in immobility time in the FST was detected (Fig. 5a; n=8 per group). As reported above, exposure to chronic stress increased the duration of immobility in the FST (p<0.05; stress+saline vs. saline treated controls, Fig. 5a; n=8 per group). Co-treatment with allopurinol attenuated the stress-related increase in immobility (p<0.05; stress+saline vs. stress +allopurinol). When body weight was recorded for the duration of the experimental period, no difference in weight gain was found when non-stressed controls were treated with either saline or allopurinol (Fig. 5b; n=8 per group). However, in response to chronic stress exposure a significant decrease in weight gain was apparent (p<0.001; stress+saline vs. saline treated controls), which was partially reversed by allopurinol treatment (p<0.05; stress+saline vs. stress+allopurinol Fig. 5b; n=8 per group).

No changes in circulating tryptophan concentrations were detected in response to stress or in response to treatment (Fig. 5c). An increase in the circulating concentration of kynurenine was detected in response to stress (p<0.01; stress+saline vs. saline treated controls, Fig. 5d). Co-treatment with allopurinol attenuated the stress-related increase in circulating kynurenine concentrations (p<0.01; stress+saline vs. stress+allopurinol, Fig. 5d). Allopurinol treatment alone failed to influence kynurenine concentrations in non-stressed controls. An increase in the kyn:tryp ratio was found after stress exposure (p<0.05; stress+saline vs. saline treated controls, Fig. 5e). Allopurinol treatment alone did not influence this ratio except when co-administered with stress, where the stress-related increase was blocked by drug treatment (p<0.05; stress+saline vs. stress+allopurinol, Fig. 5e).

**Discussion**

Exposure to acute stress provoked an increase in circulating corticosterone concentrations in association with an increase in the expression and activity of hepatic TDO. The expression and activity of hepatic TDO was sustained with chronic stress and accompanied by an increase in the expression of TDO in the frontal cortex. Increased TDO expression and activity was in turn associated with reduced circulating tryptophan and raised circulating kynurenine concentrations in tandem with an increase in immobility in the FST indicative of a depression-related behavioural phenotype. Co-administration of the TDO inhibitor allopurinol with chronic stressor exposure attenuated the stress-related increase in immobility and circulating kynurenine concentrations implicating TDO activation and KP activation in the stress and depression-related behaviour observed.

In the present investigation both acute and chronic stress exposure was accompanied by raised circulating corticosterone concentrations. Corticosterone is a known inducer of TDO (Green and Curzon, 1975; Green et al., 1975a, b, c; Young, 1981; Danesch et al., 1987; Nakamura et al., 1987; Ren and Correia, 2000). Both acute and chronic stress led to an up-regulation of TDO expression and activity in the liver, which remained elevated for 2 h post stress, returning to baseline 24 h later. These findings are in line with several other studies in which increased TDO activity was reported in the liver in response to stress (Nomura, 1965; Curzon and Green, 1969; Inamdar et al., 1972; Nemeth, 1977; Saeed and Bano, 2007; Ara and Bano, 2012). Whereas the majority of TDO found in the body is localised to the liver, some studies have reported detection of this enzyme in the brain (Miller et al., 2004; Guillemin et al., 2007;
In the present study, no effect of acute stress on cortical TDO expression was observed. However, in response to chronic stress, increased TDO expression was observed for up to 2 h following cessation of the final stressor session. To date there is no reliable method to detect TDO protein in the rodent brain and subsequently it remains unclear whether the increased expression level observed in the present study corresponds to an increase in protein level and activity of TDO in the brain. Further studies are required to fully elucidate the functional relevance, if any, of this increased cortical TDO expression.

The other major rate limiting enzyme of the KP is IDO. IDO is found ubiquitously throughout the body and is primarily activated by cytokines (Carlin et al., 1989; Fujigaki et al., 2006; Zunszain et al., 2012). As it is known that HPA axis activation can be associated with cytokine abnormalities (Connor and Leonard, 1998), it was hypothesised that increased IDO expression may also be found in the present study in response to stress. However, this was not found to be the case with similar IDO expression found in the spleen and liver of non-stressed and stressed animals. Similarly, when IDO expression was measured in the brain, no stress-induced change was detected (data not shown). This was paralleled with minimal changes to central or peripheral cytokine levels, with only a marginal stress-induced increase in IL-6 observed in the spleen and the cortex (supplementary Table 1). As recently reviewed by us (Beumer et al., 2012), several previous studies have demonstrated that both acute and chronic stress can be associated with altered immune function both in the periphery and the brain. Nevertheless, in the present study, in the absence of any indication for the induction of IDO, stress-related changes observed in tryptophan metabolism via KP activation is likely to be mediated by glucocorticoid-induced TDO.

To determine whether the stress-induced increase in hepatic TDO activity converted to alterations in circulating tryptophan and kynurenine, concentrations were determined in the serum. A short-lived induction of TDO in response to acute stress was accompanied by an increase in hepatic TDO activity yet this was insufficient to be reflected as a change in either tryptophan or kynurenine concentrations in the circulatory blood. This is in contrast to findings from a recent study in which acute stress was reported to enhance tryptophan metabolism to kynurenine (Kiank et al., 2010). However, in that investigation increased IDO expression and immunological...
abnormalities in response to stress were reported, both of which were absent in the present study.

Up-regulation of TDO following repeated exposure to stress reduced circulating concentrations of tryptophan and increased concentrations of kynurenine indicating that repeated stress exposure is required before changes in metabolism become detectable in the circulation.

As changes in tryptophan or kynurenine concentrations following chronic stress were not evident at Time 0 (immediately following cessation of stress), but appear at 2 and 24 h with tryptophan and kynurenine respectively, repeated stress promotes changes in the circulatory markers indicating activation of the kynurenine pathway. The reasons why such changes are only obtained following chronic stress are unclear but may involve stress-related adaptive changes in tryptophan transport, regulation of gene induction and/or enzymatic activity. Further molecular investigations would be required to elucidate such mechanisms.

Glucocorticoids have repeatedly been reported to increase peripheral tryptophan metabolism both in animal models and humans (Morgan and Badawy, 1989; Maes et al., 1990a, b, c; Porter et al., 2004), and while few, if any, of these studies have directly implicated TDO in changes to metabolism, it is likely to play a role. A role for the most likely alternative mechanism, the induction of indoleamine 2,3 dioxygenase, is not supported on account of the absence of changes in IDO expression in the spleen, liver or brain following acute or chronic stress. Further work will need to be undertaken to elucidate the mechanisms underlying changes in circulating tryptophan and kynurenine concentrations following chronic but not acute stress which are likely to involve changes in the regulation of the catalytic activity of TDO.

To confirm a role for TDO in the stress-induced activation of the KP and the corresponding behavioural deficits observed, the TDO inhibitor allopurinol was administered each day immediately prior to stress exposure. Co-administration of allopurinol with stress blocked stress-induced immobility in the FST. Moreover, the stress-induced increase in kynurenine was also abolished with treatment. While studies examining the effects of allopurinol on the KP are all but absent in rodents since the 1970s, a recent study assessing the effects of allopurinol on the KP in Drosophila has confirmed its ability to reduce concentrations of kynurenine and its metabolites in vivo (Al Bratty et al., 2010).

A role for TDO in the regulation of the KP, tryptophan metabolism and changes in depression-related behaviour has been largely over looked in the past few decades with research primarily focussing on immune-regulated IDO activity. However, some studies have highlighted the importance of this enzyme in the potential etiology of psychiatric disease. Kanai and co-workers have demonstrated that mice deficient for TDO display anxiolytic behaviour in various paradigms (Kanai et al., 2009b). Further studies from their lab have confirmed a key role for TDO in the regulation of neurogenesis both during development and in adulthood (Kanai et al., 2009a, b; Ohira et al., 2010). In the present study, at least with regard to chronic stress exposure, the increased depressive-like phenotype observed may therefore in part be attributable to reduced neurogenesis induced by increased TDO, and may be related to the ‘neurogenic hypothesis of
depression (Jacobs et al., 2000). In fact, stress exposure has been repeatedly linked to this hypothesis of depression with increased glucocorticoid levels directly correlating with decreased hippocampal neurogenesis and adrenalectomy in rats leading to increased cell division in this brain region (Gould et al., 1991; Cameron and Gould, 1994; McEwen, 1996; Cameron et al., 1998; Tanapat et al., 1998; Barha et al., 2011; Spanswick et al., 2011; Diniz et al., 2013; Martinez-Claros et al., 2013).

Increased TDO expression has also been found in the post-mortem brains of schizophrenic and bipolar patients (Miller et al., 2004, 2006) and seems to directly correlate with increased concentration of neurotoxic kynurenine metabolites in the same brain samples (Miller et al., 2008). In addition, several studies have demonstrated that some antidepressant treatments have the ability to reduce TDO activity (Badawy and Evans, 1982; Badawy and Morgan, 1991; Badawy et al., 1991; Walsh and Daya, 1998; Ara and Bano, 2012) and this may potentially be one additional mechanism of action by which they induce their effects. Furthermore, the effects of allopurinol observed in the present study are of interest as it is has

Fig. 5. Effect of allopurinol on behaviour in the forced swimming test (FST), body weight gain and tryptophan metabolism via the kynurenine pathway. Increased FST immobility (a) in response to chronic stress is blocked by treatment with allopurinol. Decreased body weight gain in response to chronic stress is partially reversed by treatment with allopurinol (b). No change in circulating tryptophan concentrations (c) in response to stress or allopurinol treatment. Increased circulating kynurenine concentration (d) in response to chronic stress is blocked by treatment with allopurinol. Increased kynurenine/tryptophan ratio (e) in response to chronic stress is blocked by treatment with allopurinol. Data expressed as mean±S.E.M, n=8 per group. ***p<0.001 stress+saline vs. saline treated controls; *p<0.05 stress+saline vs. saline treated controls; #p<0.05 stress+saline vs. stress+allopurinol; $p<0.05 stress+allopurinol vs. allopurinol treated controls.
been used in several studies as an adjunctive therapy for the treatment of schizophrenia (Buie et al., 2006; Dickerson et al., 2009; Liang et al., 2010; Weiser et al., 2012; Linden et al., 2013), although findings from many of these studies are as yet inconclusive.

Lack of changes in 5-HT and 5-HIAA in the present study (data not shown) would suggest that kynurenine-related metabolites are responsible for the behavioural changes observed rather than a depletion of central 5-HT. Kynurenine can readily pass through the blood brain barrier using large neutral amino acid transporters (Fukui et al., 1991), and as such the majority of kynurenine found in the brain is transported there from the periphery even in a non-immune stimulated system (Kita et al., 2002). Although the induction of cytokines in the brain was not detected in the present study, increased microglial activation has been reported in several chronic stress paradigms. It must therefore be noted that in the present study, TDO-induced kynurenine in the circulation may be transported into the brain and metabolised by microglia leading to the production of kynurenine metabolites with neuroactive and potentially neurotoxic properties (Walker et al., 2013). This may potentially occur independently or in tandem with central TDO activation and subsequently lead to changes in neuronal transmission related to behaviour. Future studies are required to clarify the putative role of individual kynurenine metabolites in models of chronic stress-induced depression.

A role for the kynurenine pathway in the pathophysiology of depression has been proposed by numerous investigators in the literature. In most cases IDO has been implicated in keeping with a role for inflammation in depression whereas the stress-related induction of hepatic TDO has not received the same degree of consideration. Both TDO and IDO are likely contributors and should be considered in tandem. A question of balance or relative contribution of either IDO or TDO is likely to depend on the presence or absence of inflammatory factors, the nature and duration of stress exposure and whether these factors are present alone or in combination. The results of the present investigation are supportive of an inclusive experimental approach to include a putative role for IDO and TDO where there is evidence of kynurenine pathway activation leading to changes in depression-related behaviours.

**Statement of Interest**

None.

**Supplementary material**

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145713001673.

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