

Hypoxanthine deregulates genes involved in early neuronal development. Implications in Lesch-Nyhan disease pathogenesis

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Abstract Neurological manifestations in Lesch-Nyhan disease (LND) are attributed to the effect of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency on the nervous system development. HPRT deficiency causes the excretion of increased amounts of hypoxanthine into the extracellular medium and we hypothesized that HPRT deficiency related to hypoxanthine excess may then lead, directly or indirectly, to transcriptional aberrations in a variety of genes essential for the function and development of striatal progenitor cells. We have examined the effect of hypoxanthine excess on the differentiation of neurons in the well-established human NTERA-2 cl.D1 (NT2/D1) embryonic carcinoma neurogenesis model. NT2/D1 cells differentiate along neuroectodermal lineages after exposure to retinoic acid (RA). Hypoxanthine effects on RA-differentiation were examined by the changes on the expression of various transcription factor genes essential to neuronal differentiation and by the changes in tyrosine hydroxylase (TH), dopamine, adenosine and serotonin receptors (DRD, ADORA, HTR). We report that hypoxanthine excess deregulate WNT4, from Wnt/ β -catenin pathway, and *engrailed* homeobox 1 gene and

increased TH and dopamine DRD1, adenosine ADORA2A and serotonin HTR7 receptors, whose over expression characterize early neuro-developmental processes.

Introduction

Lesch Nyhan disease (LND) is a neurogenetic disorder caused by complete deficiency of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) enzyme activity (Lesch and Nyhan 1964; Seegmiller et al 1967). HPRT deficiency is inherited as a recessive X-linked trait and it is caused by mutations in the *HPRT1* gene (Edwards et al 1990). HPRT catalyzes the salvage synthesis of inosine monophosphate (IMP) and guanosine monophosphate (GMP) from the purine bases hypoxanthine and guanine respectively, using 5'-phosphoribosyl-1-pyrophosphate (PRPP) as a co-substrate. Hyperuricemia accompanied by specific neurological and behavioural features characterize LND (Jinnah et al 2006; Puig et al 2001). Histopathological studies of autopsy tissue from LND cases, brain tissue from HPRT-deficient knockout mouse and neuronal cell models of HPRT deficiency have revealed no signs suggestive of a degenerative process in any brain region (Göttle et al 2014). Using voxel-based morphometry to analyse grey matter volume, Schretlen et al found that LND patients have significantly reduced, but regionally selective, brain volumes compared with healthy controls, and that smaller brain volumes are probably the result of a developmental defect, rather than a degenerative one (Schretlen et al 2013). Results of PET and autopsy studies showed a reduction in dopamine and related measures in brains of patients with LND (Ernst et al 1996; Wong et al 1996), however, the brain volume abnormalities in LND clearly show that the effects of HPRT deficiency are not restricted to the nigrostriatal dopamine pathways (Schretlen et al 2013). In previous work we have reported an imbalance of dopamine,

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adenosine and serotonin receptors in HPRT deficient cells (Garcia et al 2009; Garcia et al 2012).

To date there is strong evidence that the neurological problems in LND are caused by the effect of the HPRT deficiency on neural development of mainly, but not only, dopaminergic pathways. Growth aberrations, abnormal developmental and increased adhesion have been described in neuroblastoma and fibroblast models of Lesch Nyhan disease (Stacey et al 2000; Connolly 2001; Connolly et al 2001). In 2009, (Ceballos-Picot et al 2009) demonstrated for the first time that HPRT deficiency influences early developmental processes controlling the dopaminergic phenotype. Several reports have shown that HPRT deficiency affects functions of key transcription factors in the dopaminergic neuronal development pathway (Yeh et al 1998; Guibinga et al 2010; Kang et al 2013; Guibinga et al 2014) and microarray expression data support the fact that HPRT deficiency dysregulates Wnt/ β -catenin pathway (Kang et al 2011). However, none of these studies show the pathogenic mechanism whereby HPRT deficiency affects expression of transcription factors and neural development.

As in other enzymatic defects characterized by the toxic accumulation of the defective enzyme substrate, HPRT deficiency causes the excretion of increased amounts of hypoxanthine into the extracellular medium (Rosenbloom et al 1968; Pelled et al 1999). Based on this fact we hypothesized that HPRT deficiency produces alterations in purine metabolism including a huge hypoxanthine excess, and that this hypoxanthine excess may lead, directly or indirectly, to transcriptional aberrations in a variety of genes essential for the function and development of striatal progenitor cells. These alterations included an altered expression of dopamine, adenosine and serotonin receptors causing an imbalance in neurotransmission. In this work we have examined the effect of hypoxanthine excess on the differentiation of neurons in the well-established human NTERA-2 cl.D1 (NT2/D1) embryonic carcinoma neurogenesis model. We have tested the effect of high hypoxanthine concentration in extracellular medium on the expression of several transcription factor genes essential to neuronal differentiation and on the expression of neurotransmitters receptors. We report that hypoxanthine excess modified several transcription factors involved in early dopamine neuronal development and in pan-neuronal differentiation with increased expression of neurotransmitter receptors for dopamine, adenosine and serotonin.

Materials and methods

Cell culture

NT2/D1 is a pluripotent human testicular embryonic carcinoma cell line. NT2/D1 cells differentiate along neuroectodermal

lineages after exposure to retinoic acid (RA). The post-mitotic NT2 neurons derived from NT2/D1 cells are polarized cells that express neurofilaments, generate action potentials and calcium spikes, express, release and respond to neurotransmitters (Pleasure et al 1992).

NT2/D1 (ATCC® CRL1973™) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma Diagnostic) and 10 % (v/v) fetal bovine serum (FBS) (Gibco, Barcelona, Spain) and incubated at 37 °C in a humid atmosphere of 5 % CO₂. Subcultures were prepared by scraping. Cells from confluent cultures were dislodged from the flask surface, aspirated and dispensed into new flasks. For RA-induced differentiation, 2 x 10⁶ cells were seeded in a 75 cm² flask in DMEM with 10 % FBS with or without 25 μ M hypoxanthine (Sigma Diagnostic) and treated with 10 μ M RA (Sigma Diagnostic) twice a week for 4 weeks, as previously described (Pleasure et al 1992).

For post-mitotic NT2/D1 cells generation, RA-differentiated cells were replated again in culture dishes on Matrigel (Collaborative Research) diluted 1:60 with DMEM with 10 % FBS supplemented with 1 μ M cytosine arabinoside, 10 μ M fluorodeoxyuridine, and 10 μ M uridine. Cytosine arabinoside was continued for the first week of culture, and fluorodeoxyuridine and uridine, for the first 4 weeks (Pleasure et al 1992).

Expression of transcription factor genes essential to neuronal differentiation and neurotransmitters receptors

Cells were dislodged from the flask, aspirated and pellet by centrifugation. The cell pellet collected was washed in phosphate buffered saline (PBS) and total RNA was isolated using the QIAamp RNA Blood Mini Kit (Qiagen GmbH, d-40724, Hilden, Germany).

For gene expression quantification, total RNA was reverse transcribed into a first-strand cDNA template using the ImProm-II™ Reverse Transcriptase system (Promega, Promega Corporation, WI, USA) and oligo (dT) 15 mer as primer for RT-PCR. For all the genes we designed an adequate intron-spanning PCR assay.

Gene expression was quantified by real-time PCR in a Roche LightCycler with the use of a relative quantification method. We employed a housekeeping gene, β -actin (ACTB), as a reference gene. A control RNA was reverse transcribed and the cDNA obtained was employed as calibrator. A standard curve for each target was constructed using serial dilutions of this calibrator. The calibrator sample was assigned a concentration value of 100. Concentration was obtained from the standard curve and the results were expressed as gene target concentration/ACTB concentration ratio measured in the same sample material.

Quantification of β -actin (ACTB, NM_001101.3), type 1 dopamine receptor (DRD1, NM_000794.3), 5-hydroxytryptamine (serotonin) receptor 7 (HTR7, NM_000872.4), 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A, NM_000621.4), wingless-type MMTV integration site family, member 4 (WNT4, NM_030761.4), wingless-type MMTV integration site family, member 11 (WNT11, NM_004626.2), LIM homeobox transcription factor 1, beta (LMX1B, NM_002316.3) and *engrailed* homeobox 1 (EN1, NM_001426.3) expressions were determined using LC FastStart DNA Master SYBR Green I (Roche). A melting curve analysis was used to determine the melting temperature of the amplified products so as to ensure its specificity. Quantification of adenosine 2A type receptor (ADORA2A, NM_000675.4), adenosine 2B type receptor (ADORA2B, NM_000676.2), HPRT1 (NM_000194.2) and tyrosine hydroxylase (TH, NM_199292.2) mRNA was carried out by means of a TaqMan detection method. We employed probes from the Universal Probe Library (Roche Applied Science) and primers were designed with the Probe Finder version 2.40 for human software (Roche Diagnostics). We used TaKaRa Premix Ex Taq™ (Perfect Real Time) (TaKaRa BioEurope, France), designed for qPCR using the TaqMan probe detection method.

Data analysis

Analysis of quantification data was performed with LightCycler software. The crossing point (Cp), defined as the cycle numbers where fluorescence levels of all samples are the same, just above background, is automatically calculated by the LightCycler software by the “Second Derivative Maximum Method”. Data were expressed as the gene target concentration/ β -actin concentration ratio and presented as mean \pm SD. Statistical tests were performed using the Statview software package (SAS Institute, Inc., USA). $P < 0.05$ was considered significant.

Results

Results are expressed as mean \pm SD of at least eight determinations.

a) RA-induced differentiation and post mitotic NT2/D1 cells expression changes

Results are shown in Fig. 1. HPRT expression was not modified by RA-differentiation of NT2/D1 or in post-mitotic NT2/D1 cells.

In NT2/D1 cells RA-induced differentiation significantly increased the expression of WNT4, EN1, TH, DRD1,

ADORA2A and HTR7 with respect to undifferentiated NT2/D1 cells (Fig. 1).

On the other hand, post-mitotic NT2/D1 cells showed a significant over expression of WNT11, and LMX1B (Fig. 1) with respect to RA-differentiated cells. The expression of DRD1, ADORA2B and HTR2A were significantly increased in post-mitotic NT2/D1 cells versus RA-differentiated cells (Fig. 1).

Post-mitotic NT2/D1 cells showed a significant over expression of WNT4, WNT11 and EN1 with respect to undifferentiated cells. The expression of DRD1, ADORA2A and HTR2A were significantly increased in post-mitotic NT2/D1 cells versus undifferentiated cells (Fig. 1).

b) Hypoxanthine effects

HPRT1 expression *HPRT1* was significantly decreased by hypoxanthine in undifferentiated cells (undifferentiated cells 0.84 ± 0.49 vs. undifferentiated plus hypoxanthine 0.51 ± 0.17 , $p = 0.030$). RA-induced differentiation decrease significantly *HPRT1* expression (undifferentiated cells 0.84 ± 0.49 vs. RA-induced differentiated cells 0.47 ± 0.32 , $p = 0.012$). Hypoxanthine did not modify significantly *HPRT1* expression induced by RA (RA-induced differentiated cells 0.47 ± 0.32 , RA-induced differentiated cells plus hypoxanthine, 0.20 ± 0.06 $p = 0.065$) (Fig. 2a).

Wnt/ β -catenin pathway WNT4 expression was grossly increased by hypoxanthine in undifferentiated cells (undifferentiated cells 0.03 ± 0.03 vs. undifferentiated plus hypoxanthine 0.87 ± 0.40 , $p = 0.0039$). Hypoxanthine significantly amplified WNT4 expression induced by RA (RA-induced differentiated cells 2.60 ± 0.84 , RA-induced differentiated cells plus hypoxanthine, 4.36 ± 0.80 $p < 0.0001$) (Fig. 2b).

WNT11 expression was greatly increased by hypoxanthine in undifferentiated cells (undifferentiated cells 6.9 ± 6.3 vs. undifferentiated plus hypoxanthine 58.9 ± 12.3 , $p < 0.0001$). However, RA-induced differentiation with hypoxanthine, did not affect significantly WNT11 expression (RA-induced differentiated cells 7.6 ± 3.6 , RA-induced differentiated cells plus hypoxanthine, 7.9 ± 2.0 , $p = 0.9417$) (Fig. 2c).

LIM homeobox transcription factor 1, beta (LMX1B) and engrailed homeobox 1 (EN1) expression

LMX1B expression was significantly increased by hypoxanthine in undifferentiated cells (undifferentiated cells 4.06 ± 3.20 vs. undifferentiated plus hypoxanthine 7.48 ± 2.34 , $p = 0.0030$). RA-induced differentiation with hypoxanthine, did not affect LMX1B expression (RA-induced differentiated cells 3.59 ± 2.29 , RA-induced differentiated cells plus hypoxanthine, 4.20 ± 0.97 , $p = 0.5916$) (Fig. 2d).

EN 1 expression was not significantly affected by hypoxanthine in undifferentiated cells (undifferentiated cells 0.009 ± 0.004 vs. undifferentiated plus hypoxanthine 0.043 ± 0.012 , $p=0.6694$). Hypoxanthine addition to the culture medium significantly amplified the increase in EN 1 expression induced by RA (RA-induced differentiated cells 0.225 ± 0.149 , RA-induced differentiated cells plus hypoxanthine, 0.951 ± 0.326 , $p < 0.0001$) (Fig. 2e).

Tyrosine hydroxylase expression

TH expression was not significantly affected by hypoxanthine in undifferentiated cells (undifferentiated cells 0.13 ± 0.23 vs. undifferentiated plus hypoxanthine 0.63 ± 0.54 , $p=0.8481$). Hypoxanthine significantly amplified the increase in TH expression induced by RA (RA-induced differentiated cells 4.95 ± 7.48 , RA-induced differentiated cells plus hypoxanthine, 13.33 ± 6.25 , $p=0.0037$) (Fig. 3a).

Neurotransmitters receptor expression

DRD1 expression was greatly increased by hypoxanthine in undifferentiated cells (undifferentiated cells 1.84 ± 1.57 vs. undifferentiated plus hypoxanthine 12.4 ± 5.89 , $p=0.0005$). Hypoxanthine significantly amplified the increase in DRD1 expression induced by RA (RA-induced differentiated cells 4.87 ± 2.50 vs. RA-induced differentiated cells plus hypoxanthine, 21.2 ± 6.99 , $p < 0.0001$) (Fig. 3b)

ADORA2A expression was significantly increased by hypoxanthine in undifferentiated cells (undifferentiated cells 0.35 ± 0.43 vs. undifferentiated plus hypoxanthine 2.71 ± 1.61 , $p=0.0033$). Hypoxanthine significantly amplified the increase in ADORA2A expression induced by RA (RA-induced differentiated cells 1.72 ± 1.65 vs. RA-induced differentiated cells plus hypoxanthine, 4.21 ± 1.17 , $p < 0.0005$) (Fig. 3c)

ADORA2B expression was significantly increased by hypoxanthine in undifferentiated cells (undifferentiated cells 1.33 ± 0.84 vs. undifferentiated plus hypoxanthine 5.43 ± 1.50 , $p < 0.0001$). Hypoxanthine did not modified expression induced by RA (RA-induced differentiated cells 1.79 ± 1.02 , RA-induced differentiated cells plus hypoxanthine, 1.98 ± 0.54 , $p=0.3250$) (Fig. 3d).

HTR7 expression was not significantly affected by hypoxanthine in undifferentiated cells (undifferentiated cells 1.18 ± 0.33 vs. undifferentiated plus hypoxanthine 1.88 ± 0.61 , $p=0.2593$). Hypoxanthine significantly amplified the increase in HTR7 expression induced by RA (RA-induced differentiated cells 2.65 ± 1.58 , RA-induced differentiated cells plus hypoxanthine, 3.90 ± 0.83 , $p < 0.016$) (Fig. 3e).

HTR2A expression was not significantly affected by hypoxanthine in undifferentiated cells (undifferentiated cells 0.043 ± 0.064 vs. undifferentiated plus hypoxanthine

Fig. 1 Retinoic acid (RA)-induced differentiation and post mitotic NT2/D1 cells expression changes. UND: undifferentiated NT2/D1 cells. RA: NT2/D1 cells after 4 weeks retinoic acid induced differentiation (Pleasure et al 1992). PM: post-mitotic NT2/D1 cells generated after RA-differentiation. Cells were replated on Matrigel and supplemented with 1 μ M cytosine arabinoside, 10 μ M fluorodeoxyuridine and 10 μ M uridine (Pleasure et al 1992). Results expressed as mean \pm SD as gene target/ β -actin (ACTB) expression ratio. Wingless-type MMTV integration site family, member 4 (WNT4), wingless-type MMTV integration site family, member 11 (WNT11), LIM homeobox transcription factor 1, beta (LMX1B), *engrailed* homeobox 1 (EN1), type 1 dopamine receptor (DRD1), tyrosine hydroxylase (TH), adenosine 2A type receptor (ADORA2A), adenosine 2B type receptor (ADORA2B), 5-hydroxytryptamine (serotonin) receptor 7 (HTR7) and 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A) mRNA quantification was carried out by real-time PCR

0.012 ± 0.005 , $p=0.7983$). Hypoxanthine did not modify HTR2A expression induced by RA (RA-induced differentiated cells 0.526 ± 0.489 , RA-induced differentiated cells plus hypoxanthine, 0.523 ± 0.130 , $p=0.9804$) (Fig. 3f).

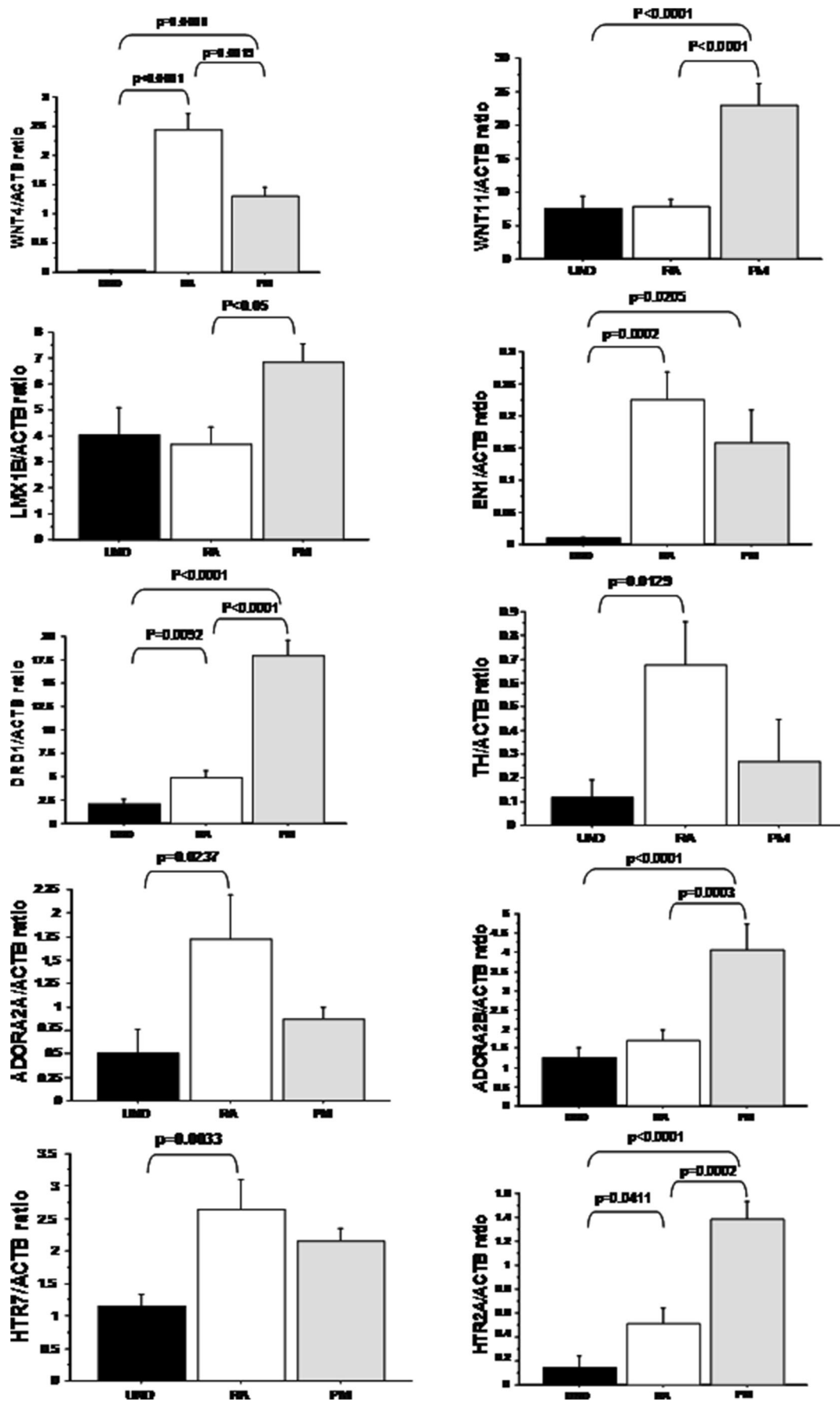
Discussion

In this study we have assessed the effect of increased hypoxanthine concentrations in the extracellular medium in NT2/D1 cell differentiation along neuroectodermal lineages after exposure to RA. Hypoxanthine effect on RA-differentiation was examined by looking at the changes in expression of various transcription factor genes which are essential to neuronal differentiation, such as WNT4 and WNT11 from Wnt/ β -catenin pathway, LMX1B and EN1.

In HPRT deficiency the reduced purine salvage results in wasting of purines, mainly hypoxanthine, that is adequately compensated by an increase in purine synthesis (Fu et al 2014). We have previously reported that hypoxanthine accumulation causes alterations in adenosine transport and function (Torres et al 2004; Prior et al 2007). In addition, hypoxanthine excess affects differentiation and proliferation in HPRT deficient neuroblastoma (Ma et al 2001).

In our study, early developmental processes in NT2/D1 cells, modelled by RA induced differentiation, was characterized by an increase in the expression of WNT4 and EN1 genes and the over expression of TH, DRD1, ADORA2A and HTR7 genes (Fig. 4a). Hypoxanthine excess significantly increased the expression of all these genes (Fig. 4b).

EN 1 is a transcription factor known to play a key role in the specialization and survival of dopamine neurons. It has been reported that HPRT deficiency increases mRNA for EN 1 in several cell models. Moreover, the increase in mRNA was accompanied by increases in *engrailed* proteins, and restoration of HPRT revert *engrailed* expression towards normal levels, demonstrating a functional relationship between HPRT and *engrailed* (Ceballos-Picot et al 2009).



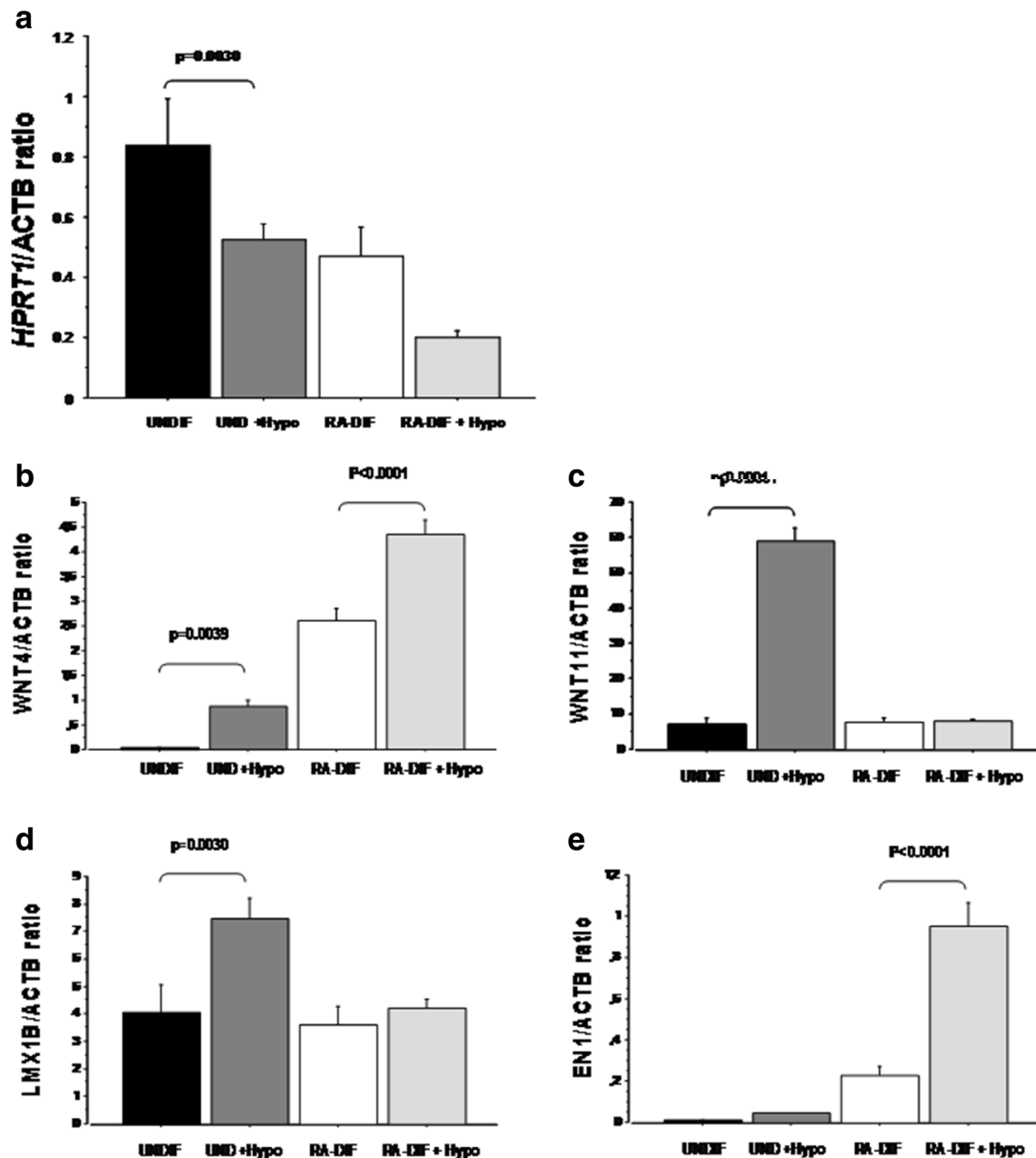


Fig. 2 Hypoxanthine expression changes on undifferentiated and retinoic acid (RA)-induced differentiated NT2/D1 cells. UNDIF: undifferentiated NT2/D1 cells. UND + Hypo: undifferentiated NT2/D1 cells plus hypoxanthine 25 μ M. RA-DIF: NT2/D1 cells after 4 weeks retinoic acid induced differentiation (Pleasure et al 1992). RA-DIF + Hypo: NT2/D1 cells after 4 weeks retinoic acid induced differentiation plus hypoxanthine 25 μ M. Results are expressed as mean \pm SD as gene

target/ β -actin (ACTB) expression ratio. **a** *HPRT1*, **b** Wingless-type MMTV integration site family, member 4 (*WNT4*), **c** wingless-type MMTV integration site family, member 11 (*WNT11*), **d** LIM homeobox transcription factor 1, beta (*LMX1B*) and **e** *engrailed* homeobox 1 (*EN1*) mRNA quantification was carried out by real-time PCR

Over-expression of *engrailed* occurred even in primary fibroblasts from patients with LND in a manner that suggested a correlation with disease severity (Ceballos-Picot et al 2009). Our results provide novel evidence that hypoxanthine excess significantly increases *EN1* expression in RA-induced differentiated NT2/D1 cells so the *HPRT* deficiency related over-expression could be mediated, at least in part, by hypoxanthine.

In *HPRT* deficient human fibroblasts and in SH-SY5Y neuroblastoma cells, microarray expression data showed that *HPRT* deficiency is accompanied by aberrations in the canonical Wnt/ β -catenin pathways (Kang et al 2013). Deregulation of the Wnt/ β -catenin pathway is confirmed by Western blot demonstration of cytosolic sequestration of β -catenin during in vitro differentiation of the SH-SY5Y cells towards the neuronal phenotype (Kang et al 2013). In this report, we have

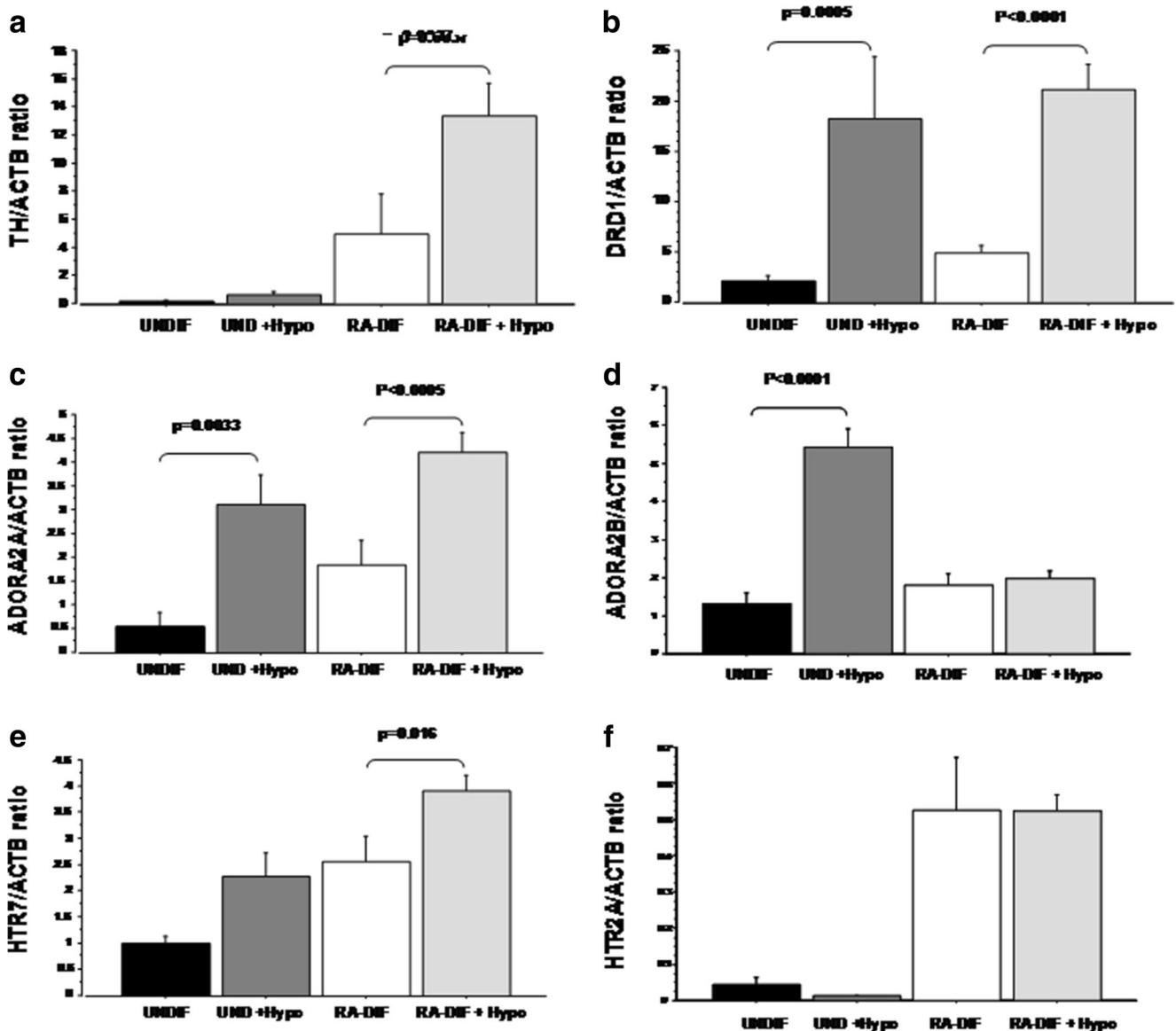


Fig. 3 Hypoxanthine expression changes on undifferentiated and retinoic acid (RA)-induced differentiated NT2/D1 cells. UNDEF: undifferentiated NT2/D1 cells. UND + Hypo: undifferentiated NT2/D1 cells plus hypoxanthine 25 μ M. RA-DIF: NT2/D1 cells after 4 weeks retinoic acid induced differentiation (Pleasure et al 1992). RA-DIF+ Hypo: NT2/D1 cells after 4 weeks retinoic acid induced differentiation plus hypoxanthine 25 μ M.

Results are expressed as mean \pm SD as gene target/ β -actin (ACTB) expression ratio. **a** tyrosine hydroxylase (TH), **b** type 1 dopamine receptor (DRD1), **c** adenosine 2A type receptor (ADORA2A), **d** adenosine 2B type receptor (ADORA2B), **e** 5-hydroxytryptamine (serotonin) receptor 7 (HTR7) and **f** 5-hydroxytryptamine (serotonin) receptor 2A (HTR2A) mRNA quantification was carried out by real-time PCR

found that hypoxanthine influences the Wnt/ β -catenin pathway by both increasing WNT11 and WNT4 expression and reinforcing the WNT4 expression induced by RA in NT2/D1 cells. Furthermore, hypoxanthine increased the expression of LMX1B, a key transcription factor, regulated by Wnt signaling, and related to the development and function of dopaminergic neurons.

RA induced differentiation in NT2/D1 cells caused an increase in the expression of the TH gene. Studies of the LND brains have revealed no signs suggestive of a degenerative process or other consistent abnormalities in any brain region.

However, neurons of the substantia nigra from the LND patients cases showed reduced immunoreactivity for TH, the rate-limiting enzyme in dopamine synthesis (Göttle et al 2014). In this study, we have found that TH expression is significantly increased when RA-differentiation is induced in NT2/D1 cells by high hypoxanthine concentration medium.

Expression of DRD1, ADORA2A and HTR7 receptor genes were increased by RA induced differentiation in NT2/D1 cells. In this study we found that DRD1 type dopamine RA-induced receptor expression is significantly increased by hypoxanthine. To a lesser extent, RA-induced ADORA2A

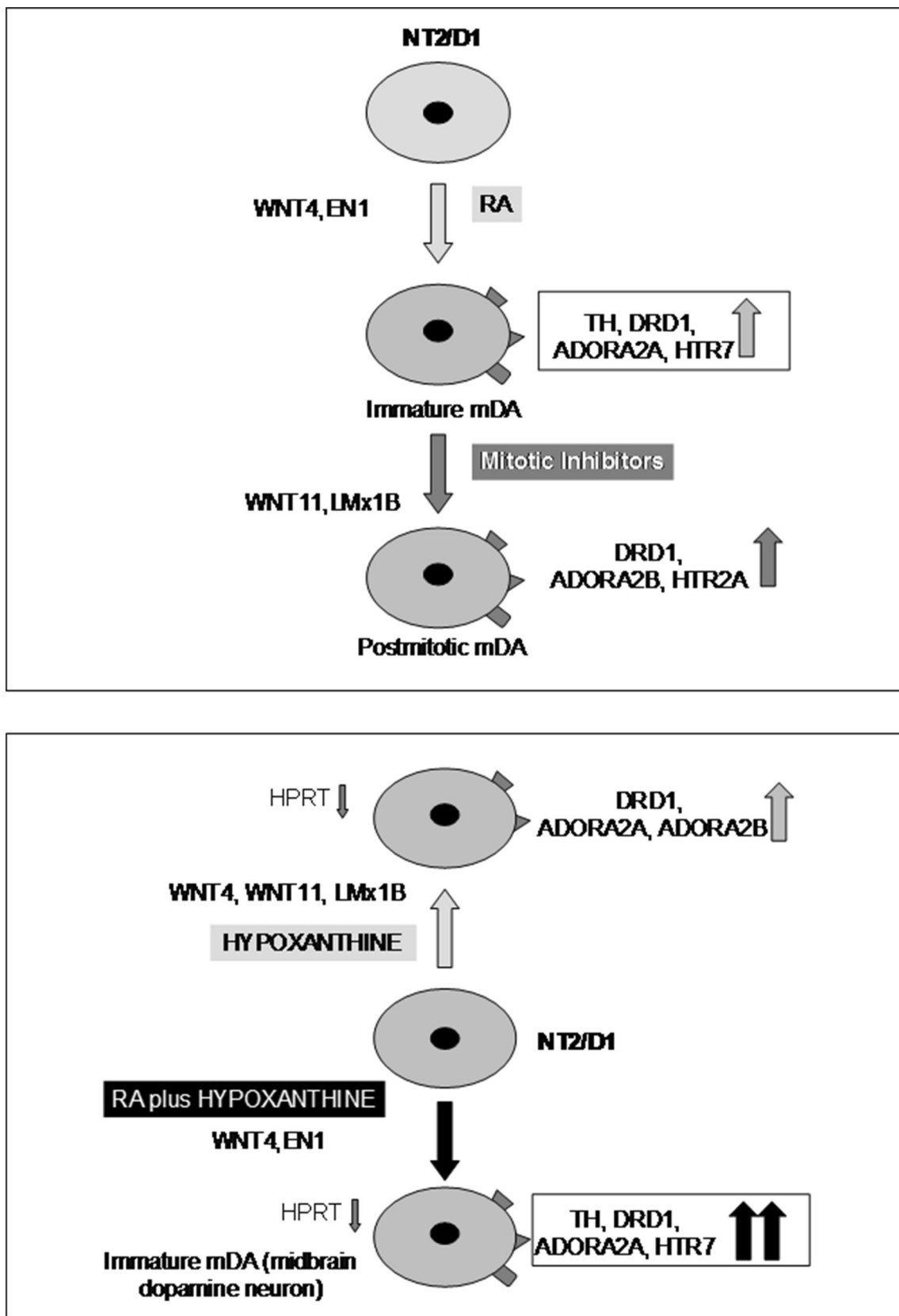


Fig. 4 Summary of changes in mRNA expression induced by a) Retinoic acid (RA)-induced differentiation and post mitotic changes and b) hypoxanthine excess with or without RA-induced differentiation

and HTR7 receptor expression are also increased by hypoxanthine. However, neither ADORA2B nor HTR2A receptor expression was modified when incubated with hypoxanthine excess. Expression of these receptors is, in our model, a hallmark of post-mitotic NT2/D1 differentiation (Fig. 4a).

One of the limitations of our study was the absence of morphological data of the hypoxanthine effect on RA-differentiated NT2/D1 cells. Although in our model hypoxanthine excess significantly decreased *HPRT1* expression, suggesting that hypoxanthine salvage was decreased in hypoxanthine treated cells, they were not fully HPRT deficient. Salvage of hypoxanthine consumes PRPP and increased IMP levels, and perhaps adenosine monophosphate and adenosine levels, and these metabolic changes may have influenced our results.

Previous reports have demonstrated that HPRT deficiency affects the functions of key transcription factors at several sites in the DA neuronal development pathway. What is not clarified is the link between HPRT deficiency and these aberrations. HPRT reutilizes purine bases and thus we hypothesized that HPRT deficiency produces an increase in the extracellular concentration of the non recycled hypoxanthine base, and this increase may then lead, directly or indirectly, to transcriptional aberrations in a variety of genes associated with neural development, including the WNT/beta catenin pathway, *LMX1B* and *EN1*. Hypoxanthine excess may also affect expression of factors related to maturation of midbrain DA neurons such as TH, the rate-limiting enzyme in dopamine synthesis, dopamine receptors, and also other neurotransmitter receptors such as adenosine and serotonin receptors. According to previous works (Torres et al 2004; Prior et al 2007), hypoxanthine excess may affect some of these genes indirectly by means of the described hypoxanthine effect on adenosine transport.

In summary, we report that hypoxanthine excess deregulated WNT4 and *EN1* genes involved in early neuronal development and increased TH and dopamine DRD1, adenosine ADORA2A and serotonin HTR7 receptors, whose over expression characterize early neurodevelopmental processes.

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Conflict of interest None.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

Details of the contributions of individual authors All authors contribute to the planning, conduct, and reporting of the work described in the article.

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