HYPOXIA INDUCES NEURITE OUTGROWTH IN PC12 CELLS THAT IS MEDIATED THROUGH ADENOSINE A2A RECEPTORS

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Abstract—Development of the nervous system is a complex process, involving coordinated regulation of diverse cellular processes including proliferation, differentiation and synaptogenesis. Disturbances to brain development such as pre- and perinatal hypoxia have been linked to behavioural and late onset of neurological disorders. This study examines the effect of hypoxia on neurite outgrowth in PC12 cells. Hypoxia not only caused a rapid induction of neurite outgrowth, but also synergistically enhanced nerve growth factor (NGF)-induced neurite outgrowth up to 24 h. Transactivation of TrkA receptors was ruled out since the TrkA inhibitor K252a did not block hypoxia-induced neurite outgrowth. Adenosine deaminase prevented hypoxia-induced neurite outgrowth indicating that the effect is mediated by adenosine. Use of the specific adenosine A2A receptor agonist CGS21680 and antagonist 8-3(chlorostyryl)caffeine demonstrated that activation of this receptor is critical for hypoxia-induced neurite outgrowth. Hypoxia-induced neurite outgrowth was blocked by the adenylate cyclase inhibitor, MDL-12,330A, indicating a role for activation of this enzyme in the pathway. Hypoxia was further shown to cause a decrease in growth-associated protein (GAP)-43 levels and a lack of induction of βIII tubulin, in contrast to NGF treatment which resulted in increased cellular levels of both of these proteins. These findings suggest that hypoxia induces neurite outgrowth in PC12 cells via a pathway distinct from that activated by NGF. Thus, exposure to hypoxia at critical stages of development may contribute to aberrant neurite outgrowth and could be a factor in the pathogenesis of certain delayed developmental neurological disorders. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: adenosine, adenylyl cyclase, βIII tubulin, GAP-43, nerve growth factor, neuronal differentiation.

Development of the nervous system is a highly complex process involving the temporal and spatial coordination of critical processes such as proliferation, migration, differentiation, synaptogenesis and programmed cell death. Vulnerable periods during development of the nervous system are sensitive to environmental insults or adverse conditions because they can interfere with these processes.

resulting in the development of late onset neurological disorders. For example, transient birth hypoxia has been shown to be a major risk factor in behavioural disorders (Berger and Garnier, 1999; El-Khodor and Boksa, 2000). Perinatal hypoxia has been linked with the development of schizophrenia in humans (Davies et al., 1998; Boksa and El-Khodor, 2003) although the biochemical mechanism underlying this is unknown. One current theory of the neuropathology of schizophrenia is that it is a developmental disorder of ‘neural connectivity’ (Benes, 2000; Penn, 2001) and as such it may be the result of interference with normal ontogeny of developmental processes such as neuronal differentiation in the nervous system. However, the mechanisms by which this occurs have not been elucidated and whether or not hypoxia plays a role in aberrant neuronal development is unknown.

Cellular exposure to hypoxic conditions results in alterations in gene transcription that promote expression of proteins involved in the glycolytic pathway and repress genes encoding components of the electron transport chain (Webster, 1987; Semenza et al., 1994). Activation by hypoxia of the basic helix–loop–helix transcription factor, hypoxia-inducible factor-1 (HIF-1), is largely responsible for these changes in gene transcription (Semenza and Wang, 1992; Wang et al., 1995). HIF-1 activation occurs through stabilisation of one of its two subunits, HIF-1α (Huang et al., 1996), which then dimerises with the constitutively expressed HIF-1 (Kallio et al., 1997).

Another important cellular response to low oxygen is altered adenosine metabolism with a consequent increase in the rate of adenosine release (Kobayashi et al., 2000). Adenosine has been shown to modulate neuronal function via adenosine receptors (Daval et al., 1991). To date, four adenosine receptors have been cloned and characterised (A1, A2A, A2B and A3) (Olah and Stiles, 1995) and have been suggested to influence neuronal differentiation, migration, synaptogenesis and survival (Marangos et al., 1982; Weaver, 1993, 1996; Paes-De-Carvalho, 2002; Stone, 2002; Aden et al., 2003; Schwarzschild et al., 2003). Rat pheochromocytoma (PC12) cells express adenosine A1, A2A and A2B receptors (Arslan et al., 1999). These cells have the ability to differentiate to a sympathetic neuron-like phenotype in response to the neurotrophic factor nerve growth factor (NGF; Greene and Tischler, 1976). Binding of NGF to TrkA receptors on PC12 cells induces receptor autophosphorylation and initiates the mitogen-activated protein kinase (MAPK) cascade that leads to neuronal differentiation of PC12 cells (Pang et al., 1995). Activation of adenosine A2A receptors, which are positively coupled to adenylyl cyclase (Ribeiro et al., 1999),...
has been shown to potentiate NGF-induced neurite outgrowth in PC12 cells (Cheng et al., 2002). The aim of the present study was to examine the effect of hypoxia on neurite outgrowth and to further examine the effect of hypoxia on NGF-mediated neurite outgrowth. This investigation was carried out using PC12 cells which are a useful model to study neurite outgrowth.

**EXPERIMENTAL PROCEDURES**

**Cell culture**

PC12 cells (ECACC no. 88022401) were routinely maintained in RPMI 1640 medium supplemented with 5% foetal calf serum, 10% heat-inactivated horse serum, 50 U/ml penicillin, 50 mg/ml streptomycin and 2 mM L-glutamine (full medium). Cells were routinely subcultured every 4–5 days.

**Treatment of cells**

PC12 cells were plated onto glass coverslips coated with poly-L-lysine (0.001%) at a density of 7.5 × 10⁴ cells/33 mm dish in 2 ml of medium. Prior to experimental treatment the cells were grown overnight in full medium, after this time the medium was changed and replaced with RPMI 1640 media supplemented with 1% horse serum, 50 U/ml penicillin, 50 mg/ml streptomycin and 2 mM L-glutamine (full medium). NGF treatment (2.5 μg/ml NGF from Alomone Laboratories, Jerusalem, Israel) was carried out by including 50 ng/ml NGF in low serum medium for the required time. The medium containing NGF was changed daily. Hypoxia treatment was achieved using the InVivo2 hypoxia workstation (Ruskinn Technology, Dublin, Ireland) set to maintain conditions of 0.5% O₂. For chemical treatments stocks of adenosine (Sigma-Aldrich Ireland Ltd., Dublin, Ireland), 8-(3-chlorostyryl)caffeine (CSC; Sigma) and MLD-12.330A (Calbiochem, Merck Biosciences Ltd., Nottingham, UK) were made up in water whilst 2-[4-(2-carboxyethyl)phenethylamino]-5-N-ethylcarboxamidoadenosine (CGS21680) and SB202190 (Calbiochem) were dissolved in dimethylsulphoxide (DMSO). Samples containing 20–25 μg protein were separated on 12% SDS–polyacrylamide gels at 100 V for 100 min. Western blot analysis was carried out for 3 h at room temperature. Detection of bands was achieved using either horseradish peroxidase-labelled goat anti-mouse (diluted 1:10,000 in PBS, 1% BSA) or for anti-p38 MAPK (obtained from Biosource UK, Nivelles, Belgium) diluted 1:1000 in PBS containing 0.2% Tween-20 and 3% BSA. Incubation with horseradish peroxidase-labelled goat antimouse (diluted 1:10,000 in PBS, 1% BSA and 0.2% Tween-20) was carried out for 3 h at room temperature. Detection of bands was carried out using Supersignal West Pico chemiluminescent kit (Pierce; Medical Supply Company Ltd., Dublin, Ireland).

**Statistical analysis**

Results are expressed as means ± S.E.M. Statistical analysis was made using Student unpaired t-test or repeated measures ANOVA followed by appropriate post hoc tests.

**RESULTS**

**Hypoxia induces neurite outgrowth in PC12 cells**

Exposure of PC12 cells to hypoxia (0.5% O₂) for 3 days was found to induce neurite outgrowth (Fig. 1A). The cells underwent morphological changes that were comparable to those induced by NGF (Fig. 1A) which is a well-known inducer of neuronal differentiation in these cells (Greene et al., 2000).
Cells exposed to hypoxia became flattened and extended long processes (Fig. 1A). Naive PC12 cells did not show any basal neurite outgrowth. A combination of hypoxia and NGF treatment also stimulated neurite outgrowth (Fig. 1A). The induction of neurite outgrowth by hypoxia was quantified over 0–4 days and compared with that induced by NGF treatment. There was a time-dependent increase in neurite outgrowth as a result of hypoxia exposure (Fig. 1B) reaching a maximum of 13.3±1.4% after 3 days in hypoxia. This was significantly lower than the level of outgrowth due to NGF treatment, which reached 27.5±2.2% after 3 days (Fig. 1B). Prolonged exposure of the cells to hypoxia beyond 3 days caused a decrease in the level of neurite-bearing cells which is in contrast to the continued increase observed with NGF treatment (Fig. 1B). This was due to a decrease in cell survival (measured using an MTT assay) in cultures exposed to hypoxia for periods longer than 2 days (Fig. 1C).

Lack of involvement of HIF-1 in hypoxia-induced neurite outgrowth

Many intracellular responses to hypoxia involve changes in gene expression that are coordinately regulated by HIF-1 (Semenza and Wang, 1992; Wang et al., 1995). Treatment of the cells with CoCl₂ under normoxic conditions is known to mimic many elements of the hypoxic response (Huang et al., 1996) through its ability to stabilise HIFα (Kallio et al., 1997). In order to examine whether HIF-1 activation played a role in neurite outgrowth, PC12 cells grown in normoxic conditions were exposed to 0–100 μM CoCl₂ for 3 days and the level of neurite outgrowth determined. None of the concentrations tested induced neurite outgrowth (data not shown) and in fact, higher concentrations (≥50 μM) caused cell degeneration (data not shown).

Hypoxia-induced neurite outgrowth is not mediated through TrkA

In order to examine whether hypoxia-induced neurite outgrowth was mediated via activation of the TrkA receptor, the effect of K252a (a potent inhibitor of the tyrosine kinase activity of TrkA) was assessed. K252a (50 nM and 100 nM) was found to inhibit NGF-induced neurite outgrowth (Table 1). In contrast, K252a did not affect hypoxia-induced neurite outgrowth (Table 1). This excludes an involvement of TrkA activation in hypoxia-induced neurite outgrowth.

Effect of hypoxia on GAP-43 and βIII tubulin expression

GAP-43 is a neuronal specific axonal marker that is upregulated following NGF-mediated neurite outgrowth (Das et al., 2004) and class III β tubulin is a neuronal specific isoform of β tubulin (Lee et al., 1990). Since it appears that hypoxia and NGF cause neurite outgrowth via different mechanisms, it was decided to examine intracellular markers of neuronal differentiation. Western blot analysis showed a time-dependent increase in GAP-43 expression.
Table 1. Effect of K252a on induction of induction of neurite outgrowth by hypoxia and NGFa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neurite outgrowth % cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGF alone</td>
<td>33.0±5.3</td>
</tr>
<tr>
<td>NGF plus 50 nM K252a</td>
<td>16.9±2.9</td>
</tr>
<tr>
<td>NGF plus 100 nM K252a</td>
<td>4.3±1.5**</td>
</tr>
<tr>
<td>Hypoxia alone</td>
<td>9.3±2.9</td>
</tr>
<tr>
<td>Hypoxia plus 50 nM K252a</td>
<td>12.1±4.3</td>
</tr>
<tr>
<td>Hypoxia plus 100 nM K252a</td>
<td>12.1±3.4</td>
</tr>
</tbody>
</table>

* PC12 cells were pretreated with K252a (50 nM or 100 nM) for 30 min prior to exposure to hypoxia or NGF for 3 days. Neurite outgrowth was quantified. The data are the mean±SEM of three independent experiments. The data were analysed using ANOVA with Tukey-Kramer posthoc test.

Table 2. Effect of adenosine deaminase on induction of induction of neurite outgrowth by hypoxiaa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neurite outgrowth % cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia alone</td>
<td>15.35±0.71</td>
</tr>
<tr>
<td>Hypoxia plus ADA</td>
<td>0.91±0.06***</td>
</tr>
</tbody>
</table>

* PC12 cells were pretreated with 1 unit/ml adenosine deaminase (ADA) for 30 min prior to exposure to hypoxia for 2 days. Neurite outgrowth was quantified. The data are the mean±SEM of four independent experiments. The data were analysed using Student's unpaired t test.

**P<0.01 versus no adenosine deaminase.

Hypoxia-induced neurite outgrowth is mediated through A2A receptor activation

Adenosine has previously been shown to be released from cells upon exposure to hypoxia (Kobayashi et al., 2000). A role for exogenous adenosine in hypoxia-induced neurite outgrowth was examined by incubating PC12 cells with adenosine deaminase during 2 day exposure to hypoxia. Adenosine deaminase was found to completely block neurite outgrowth by hypoxia (Table 2; P<0.001).

In particular, stimulation of the adenosine A2A receptor has been shown to induce differentiation in PC12 cells (Charles et al., 2003). Therefore, it was decided to examine whether the adenosine A2A receptor was involved in hypoxia-induced neurite outgrowth. PC12 cells were pretreated with 0–20 nM CSC (a specific antagonist of the adenosine A2A receptor) for 0.5 h prior to exposure to hypoxia for 3 days. There was a dose-dependent decrease in neurite outgrowth (Fig. 3A). The proportion of cells bearing neurites in cultures exposed to hypoxia alone decreased significantly in the presence of CSC (Fig. 3A; P<0.01, n=3). To verify the involvement of the adenosine A2A receptor in neurite outgrowth by hypoxia, PC12 cells were exposed to a range of concentrations of adenosine or the specific adenosine A2A receptor agonist (CGS21680) under normoxic conditions and the extent of neurite outgrowth was determined. Both of these adenosine receptor agonists caused induction of neurite outgrowth (Fig. 3B, C). The maximum level of neurite-bearing cells was 8.5% for 50 μM adenosine (P<0.01, n=3) and 5.1% for 50 nM CGS21680 (P<0.001, n=3).

Adenosine A2A receptor activation is known to stimulate adenylate cyclase (Schulte and Fredholm, 2003). In order to examine whether hypoxia-induced neurite outgrowth involved activation of this enzyme the effect of the adenylate cyclase inhibitor, MDL-12,330A, on hypoxia-induced neurite outgrowth was determined. MDL-12,330A was found to significantly inhibit the effect of hypoxia (Fig. 4).

Hypoxia synergistically enhances NGF-induced neurite outgrowth

Previous reports have shown that purines, e.g. guanosine (Gysbers and Rathbone, 1996a,b) and adenosine (Huffaker et al., 1984; Muroi et al., 2004), can synergistically enhance NGF-induced neurite outgrowth in PC12 cells. Although there...
did not appear to be any enhancement of the effect of hypoxia on NGF-induced neurite outgrowth at 2, 3 or 4 days (Fig. 1B), at the earliest time tested (1 day) neurite outgrowth due to the combination of the two stimuli was slightly higher than that due to NGF alone. This suggested that hypoxia might enhance the initiation of neurite outgrowth due to NGF. Therefore, it was decided to examine the effect of hypoxia on NGF-induced neurite outgrowth at earlier times (0–24 h). It was found that at the earlier times hypoxia synergistically enhanced the level of neurite outgrowth due to NGF (Fig. 5A). The combination of the two stimuli caused a rapid increase in the proportion of cells with neurites. The synergistic effect of hypoxia was completely antagonised by CSC (Fig. 5B), demonstrating that this effect is mediated through the adenosine A2A receptor.

In order to confirm that synergy between hypoxia and NGF is through the adenosine A2A receptor the induction of neurite outgrowth by a combination of either NGF plus adenosine or NGF plus CGS21680 was compared with the expected outgrowth if the effects were not synergistic (i.e. additive). The combination of NGF plus adenosine induced neurite outgrowth that was greater than that expected when the values for separate NGF and adenosine treatment were added (Fig. 5C); however, this was not statistically significant. Combined treatment with NGF plus CGS21680 led to neurite outgrowth that was significantly different from that expected when the values for separate NGF and CGS21680 treatment were added (Fig. 5C, \( P < 0.05 \)). This indicates that there is synergy between NGF and adenosine A2A receptor activation in the induction of neurite outgrowth.

**Involvement of p38 MAPK in hypoxia-induced neurite outgrowth**

There is evidence that diverse signalling pathways including activation of p38 MAPK can lead to induction of neurite outgrowth (Morooka and Nishida, 1998; Iwasaki et al., 1999; Hansen et al., 2000; Choi et al., 2001; Ishii et al., 2001; Takeda and Ichijo, 2002). In the present study, both NGF and hypoxia were found to stimulate a rapid increase in the level of phosphorylated p38 MAPK (Fig. 6A). Hypoxia-induced phosphorylation occurred slightly earlier than that due to NGF treatment (3 h compared with 6 h). In order to examine the role of p38 MAPK in hypoxia-induced neurite outgrowth, a specific inhibitor, SB202190, was included during exposure of the cells to hypoxia. However,
this combination of treatment caused the cells to die (data not shown). Since the adenosine A2A receptor has been shown to mediate the effect of hypoxia (Fig. 3), the effect of SB202190 on CGS21680-induced neurite outgrowth was examined. It was found that SB202190 inhibited CGS21680-induced neurite outgrowth in a dose-dependent manner, with essentially complete inhibition occurring at 2.5 μM SB202190 (Fig. 6B; \( P < 0.01 \)).

**DISCUSSION**

The data presented in this study demonstrate that exposure to hypoxia stimulates a rapid induction of neurite outgrowth in PC12 cells. An involvement of HIF-1 was ruled out since CoCl2, which chemically stabilises HIF-1α (Kallio et al., 1997), did not induce neurite outgrowth. In contrast, hypoxia-induced neurite outgrowth in PC12 cells was blocked by the adenosine A2A receptor antagonist, CSC, and could be mimicked by adenosine and the CGS21680 (a specific adenosine A2A receptor agonist), indicating that the effect is mediated through adenosine A2A receptors. Moreover, activation of adenosine A2A receptors is known to activate adenylate cyclase (Ribeiro et al., 2002) and the present study shows that inhibition of adenylate cyclase with MDL-12,330A blocks hypoxia-induced neurite outgrowth. Transactivation of Trk neurotrophin receptors (in the absence of NGF) by activation of adenosine A2A receptors has previously been reported (Lee and Chao, 2001; Piiper et al., 2002). However, despite the demonstrated involvement of the adenosine A2A receptor, hypoxia-induced neurite outgrowth was not suppressed by a selective inhibitor of TrkA, indicating that transactivation of TrkA by adenosine A2A receptors could not provide a mechanism to explain how hypoxia caused neurite outgrowth in the present study.

Purines such as adenosine have increasingly been recognised as important intercellular trophic signalling molecules in the nervous system, influencing cell growth, differentiation and death. Adenosine (Huffaker et al., 1984; Braumann et al., 1986; Muroi et al., 2004), inosine (Braumann et al., 1986) and guanosine (Gysbers and Rathbone, 1992, 1996a,b) have all been reported to induce neurite outgrowth or to enhance NGF-induced neurite outgrowth. Induction of neurite outgrowth in PC12 cells by the bacterial nucleoside N6-methyldeoxyadenosine has been demonstrated to be mediated by adenosine A2A receptors (Charles et al., 2003). Moreover, chronic hypoxia has been reported to enhance adenosine release in PC12 cells by

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**Fig. 5.** Co-treatment with hypoxia enhances NGF-induced neurite outgrowth. (A) PC12 cells were treated with hypoxia, NGF or combined hypoxia/NGF for up to 24 h and the levels of neurite outgrowth were examined. Values are the means±S.E.M. of three independent determinations. The data were analysed using ANOVA with Dunnett’s post hoc test. * \( P < 0.05 \), ** \( P < 0.01 \), versus untreated cells. (B) PC12 cells were treated with CSC (5 nM) for 30 min prior to exposure to NGF, hypoxia or combined hypoxia/NGF for 9 h. The levels of neurite outgrowth were determined. Values are the mean±S.E.M. of four independent experiments. The sum of the values obtained from separate NGF and Ado or NGF plus CGS treatments (separate) were compared with the actual values that were obtained from combined NGF plus Ado or NGF plus CGS treatments (combined) using Student’s t-test. * \( P < 0.05 \) versus the sum of separate treatments.
Exposure to hypoxia in the presence of NGF was found to accelerate the initial phase of induction of neurite outgrowth. At early times of exposure to hypoxia and NGF there is a synergistic effect by the two treatments but this effect is only transient and is ameliorated by 2 days. It is likely that this initial synergy is mediated by cAMP since the adenosine A2A receptor is positively coupled to adenylate cyclase leading to an increase in intracellular cAMP (Schulte and Fredholm, 2003). Synergistic induction of neurite outgrowth was also observed at early times of combined CGS21680 and NGF treatment. A similar result was obtained at early times of treatment with adenosine and NGF; however, it was not statistically significant. These findings are in agreement with other groups who have reported synergistic enhancement of NGF-induced neurite outgrowth by adenosine (Muroi et al., 2004), the adenosine analog 5′-N-ethylcarboxamidoadenosine (Gurvoff et al., 1981) and intracellular cAMP (Heidemann et al., 1985; Charles et al., 2003).

Differential regulation of GAP-43 and βIII tubulin expression by NGF and hypoxia was observed in the present study. NGF induced an upregulation in the expression of both GAP-43 and βIII tubulin. On the other hand, hypoxia caused a downregulation in GAP-43 and did not induce βIII tubulin expression. GAP-43 has a role in pathfinding during neurite outgrowth and is preferentially localized in the growth cone and elongating axon of developing neurons. Neurons growing in vivo or in vitro express high levels of GAP-43 at the beginning of neurite outgrowth (Perrone-Bizzozero et al., 1986; Meiri et al., 1988; Costello et al., 1990; Dani et al., 1991). Beta III tubulin is recognized as a marker of neuronal cells and its upregulation is associated with neuronal differentiation (Menezes and Luskin, 1994). NGF is well known to increase GAP-43 expression in PC12 cells during differentiation (Costello et al., 1990). This NGF-induced increase in GAP-43 expression is due to protein kinase C-dependent stabilization of the GAP-43 mRNA (Perrone-Bizzozero et al., 1993). The divergent regulation of GAP-43 expression and neurite outgrowth has been demonstrated in various systems. For example, neurite outgrowth in the absence or repression of GAP-43 expression has been reported in cultured adult rat dorsal root ganglion neurons (Andersen et al., 2000a), the serotonergic CNS cell line RN46A (Andersen et al., 2000b) and certain clones of PC12 cells (Baetge and Hammang, 1991; Burry and Perrone-Bizzozero, 1993). Neurite outgrowth without increased GAP-43 is associated with cAMP signaling (Burry and Perrone-Bizzozero, 1993; Andersen et al., 2000a,b). These reports support the present finding that GAP-43 expression and neurite outgrowth can be uncoupled and that hypoxia-induced neurite outgrowth is mediated by activation of adenosine A2A receptors coupled to adenylate cyclase. However, the fact that cells fail to express βIII tubulin when treated with hypoxia suggests that the outgrowths are qualitatively different from those induced by NGF. To our knowledge this is the first demonstration of neurite outgrowth in PC12 cells in the absence of an induction of βIII tubulin.
Activation of ERK and p38 MAP kinase pathways are required for NGF-induced neuronal differentiation in PC12 cells (Cowley et al., 1994; Morooka and Nishida, 1998). Cyclic AMP has been reported to induce neurite outgrowth and differentiation through p38 MAPK activation (Hansen et al., 2000) or through a pathway dependent on protein kinase A and phosphatidylinositol-3 kinase activity (Sanchez et al., 2004). The role of p38 MAPK in hypoxia-induced neurite outgrowth was investigated, since activation of the cAMP pathway has previously been reported to induce a rapid, dose-dependent phosphorylation and activation of this enzyme (Hansen et al., 2000). The present study demonstrates that both hypoxia and NGF induce phosphorylation of p38 MAPK suggesting that the pathways are not completely divergent. Hypoxia-induced neurite outgrowth was inhibited by SB202190 indicating that activation of p38 MAPK is essential in this process. Taken together, these findings are in agreement with those of Hansen et al. (2000) who show that induction of neurite outgrowth by cAMP requires p38 MAPK activation.

Correct pathfinding and connection formation in the brain is critical for the normal development and functioning of the brain. Exposure of the developing foetus to hypoxia during the pre- or perinatal period is linked to long term neurological deficits and the development of behavioural abnormalities (Davies et al., 1998; Berger and Garnier, 1999; El-Khodor and Boksa, 2000; Boksa and El-Khodor, 2003). Based on the present findings these effects of hypoxia could be mediated by adenosine A2A receptor-dependent induction of inappropriate neurite outgrowth or enhancement of neurotrophin-induced neurite outgrowth, leading to aberrant pathfinding and connection formation. Neurites that are induced by hypoxia are biochemically distinct from those induced by NGF in that hypoxia fails to induce GAP-43 or βIII tubulin expression. This suggests that these neurites may display defective pathfinding capacity since this protein is preferentially localised in the growth cone and elongating axon of developing neurons. Thus, the present findings may provide a mechanism of how exposure to hypoxia during critical periods of development of the nervous system can lead to long term neurological abnormalities. Further work is necessary to investigate the effects of hypoxia on differentiation in primary neuronal cell cultures.

Acknowledgments—This work was financially supported by the Health Research Board of Ireland and the Millenium Fund, National University of Ireland, Galway.

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(Received 15 November 2004) (Available online 28 January 2005)