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Research Report

Brain-derived neurotrophic factor (BDNF) and TrkB in the piglet brainstem after post-natal nicotine and intermittent hypercapnic hypoxia

Samantha Tang[a], *Rita Machaalani*[b,c,*], *Karen A. Waters*[a,b,c]

[a]*Department of Paediatrics and Child Health, The University of Sydney, NSW 2006, Australia*
[b]*Department of Medicine, Room 206, Blackburn Building, D06, The University of Sydney, NSW 2006, Australia*
[c]*The Children's Hospital, Westmead Sydney, NSW 2145, Australia*

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) and its receptor TrkB play a significant role in the regulation of cell growth, survival and death during central nervous system development. The expression of BDNF and TrkB is affected by noxious insults. Two insults during the early post-natal period that are of interest to our laboratory are exposure to nicotine and to intermittent hypercapnic hypoxia (IHH). Piglet models were used to mimic the conditions associated with the risk factors for the sudden infant death syndrome (SIDS) including post-natal cigarette smoke exposure (nicotine model) and prone sleeping where the infant is subjected to re-breathing of expired gases (IHH model). We aimed to determine the effects of nicotine and IHH, alone or in combination, on pro- and rhBDNF and TrkB expression in the developing piglet brainstem. Four piglet groups were studied, with equal gender ratios in each: control ($n=14$), nicotine ($n=14$), IHH ($n=10$) and nic+IHH ($n=14$). Applying immunohistochemistry, and studying six nuclei of the caudal medulla, we found that compared to controls, TrkB was the only protein significantly decreased after nicotine and nic+IHH exposure regardless of gender. For pro-BDNF and rhBDNF however, observed changes were more evident in males than females exposed to nicotine and nic+IHH. The implications of these findings are that a prior nicotine exposure makes the developing brainstem susceptible to greater changes in the neurotrophic effects of BDNF and its receptor TrkB in the face of a hypoxic insult, and that the effects are greater in males than females.

* *Corresponding author*. Department of Medicine, Room 206, Blackburn Building, DO6, University of Sydney, NSW 2006, Australia. Fax: +61 2 9550 3851.
E-mail address: ritam@med.usyd.edu.au (R. Machaalani).

Abbreviations: $5HT_{1A}R$, serotoninergic receptors 1A; $5HT_{2A}R$, serotoninergic receptors 2A; APTES, 3-amino-prophyltriethoxysilane; BDNF, Brain-derived neurotrophic factor; BDNF–/–, Brain-derived neurotrophic factor knockout; Cun, Cuneate nucleus; DAB, Diamino-benzidine/H_2O_2; DMNV, Dorsal motor nucleus of the vagus; DPX, di-*n*-butylphthalate in Xylene; GCMS, Gas chromatography mass spectrometry; GG, Genioglossus; Grac, Gracile nucleus; GST-fusion, Glutathione-S-transferase-fusion; IHC, Immunohistochemistry; IHH, Intermittent hypercapnic hypoxia; ION, Inferior olivary nucleus; KLH, Keyhole limpet hemocyanin; nAChRs, nicotinic acetylcholine receptors; NHS, Normal horse serum; Nic, Nicotine; Nic+IHH, Combined nicotine and IHH; NTS, Nucleus of the tractus solitarus; OSA, Obstructive sleep apnea; PBS, Phosphate buffered saline; PC12, Pheochromocytoma-12; rhBDNF, Recombinant human brain-derived neurotrophic factor; SIDS, Sudden Infant Death Syndrome; TrkB, Tyrosine kinase B; XII, Hypoglossal nucleus

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1. Introduction

Growth factors are required for the normal development and functioning of the nervous system. One group of growth factors includes the neurotrophins, a family of secretory proteins, consisting of nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5. Of the neurotrophins, BDNF plays a significant role in the promotion of normal nervous system development, and in regulating cell survival and apoptosis. BDNF is very important in the development of the system for regulating respiration (Balkowiec and Katz, 1998; Erickson et al., 1996). In particular, BDNF knockout (–/–) mice show severe depression of respiratory frequency and minute ventilation, with a loss of the hypoxic ventilatory drive (Balkowiec and Katz, 1998; Erickson et al., 1996).

BDNF exists in two forms, pro-BDNF and mature-BDNF (Heymach and Shooter, 1995). Initial translation is to pro-BDNF, a 30–35 kDa precursor to mature-BDNF which is formed by cleavage of either furin or pro-convertase. Pro- and mature-BDNF are distinct ligands and interact primarily with different receptor systems, $p75^{NTR}$ and TrkB receptors respectively, which determine their functions. When pro-BDNF binds with the $p75^{NTR}$ receptor it mediates cell death (Friedman, 2000), whereas the interaction between mature-BDNF and its TrkB receptor, mediates neuronal survival, differentiation and synaptic function (Patapoutian and Reichardt, 2001). The opposing actions of the BDNF systems have become of much interest in the control of cell survival in neurodegenerative pathologies (Nosheny et al., 2005).

Sudden infant death syndrome (SIDS), the leading cause of post-neonatal deaths in developed countries, is thought to be due to failure to respond adequately to a significant respiratory challenge such as re-breathing when in the prone sleep position. Infants dying from SIDS have increase expression of apoptotic markers, as well as altered neurotransmitter and neuronal receptor expressions in respiratory related regions of the brainstem (Kinney et al., 1992; Machaalani et al., 2007; Oehmichen et al., 1998; Sparks et al., 1996; Waters et al., 1999). The presence of these changes is consistent with the hypothesized abnormalities of respiratory control. Given the strong role of the BDNF system in respiratory control, we hypothesized that the disruption of the respiratory pathways in SIDS infants would involve demonstratable changes in BDNF expression.

Although the cause(s) of SIDS remains unknown, risk factors have been identified. The two major modifiable factors that have been identified are the prone sleeping position and cigarette smoke exposure. In order to more fully understand the mechanisms by which these risk factors contribute to a SIDS death, our laboratory developed piglet models for these two risk exposures. The first involves exposure to intermittent hypercapnic hypoxia (IHH) (Waters and Tinworth, 2001), the second, to post-natal nicotine (Machaalani et al., 2005), and the third exposes piglets to a combination of IHH and nicotine (Machaalani and Waters, 2006). The IHH model mimics the re-breathing episodes experienced by infants during prone sleeping or obstructive sleep apneas (OSA) (Waters and Tinworth, 2001). The nicotine model mimics passive cigarette smoke exposure experienced by the infant (Machaalani et al., 2005). Nicotine was the chosen substance of cigarette smoke exposure based on data that nicotine levels were high in infants from smoking mothers (it was estimated that a 6 kg infants would breath in 7–600 ng/h/kg of nicotine via passive smoking (Kubin et al., 1998)), and that nicotine is the most neuroteratogenic agent in cigarettes (Slotkin, 1998).

Piglets show many similarities to the human infant in their physiological and neurological processes. The brain growth spurt starts at mid-gestation in both the human and piglet, and the two species show equivalent degrees of myelination and cellularity at different stages of growth (Dobbing and Sands, 1979). The piglets used in this study were aged 13–14 days, which is equivalent to the human age of 2–4 months, and is coincident with the peak occurrence of SIDS deaths (Australian Bureau of Statistics., 2000).

Current literature is limited with regard to the effects of hypoxia or nicotine on BDNF and/or TrkB expression. Studies have mainly focused on the hippocampal (Aleisa et al., 2006; French et al., 1999; Kenny et al., 2000; Maggio et al., 1998; Narumiya et al., 1998) and cerebellar (Scheepens et al., 2003) brain regions. There are no studies on the brainstem during the newborn period, with the exception of our own study that compared the expression of mature-BDNF after different durations of IHH exposure (Peiris et al., 2004). Other studies examining the effects of hypoxia or nicotine exposures on BDNF and TrkB expression used western blotting techniques, which has limited capacity to identify changes in expression at regional and cellular levels. Regional and cellular changes are best identified by immunohistochemistry (IHC), which was the method used in this study.

In this study we extend our previous investigation to examine the effects of nicotine exposure, separately and combined with IHH, on mature-BDNF, pro-BDNF and TrkB expression by IHC. We hypothesized that independent exposure to either nicotine or IHH will increase expression of both pro- and rhBDNF, and will decrease TrkB receptor expression in the brainstem medullary nuclei. We also hypothesized that combined exposure to IHH and nicotine would amplify the changes in expression of pro- and rhBDNF and the TrkB receptors. Increased expression of pro-BDNF would support its role in increased apoptosis. Increased expression of rhBDNF would support an increased need for neuronal protection. Decreased TrkB would suggest reduced cell survival and increased apoptosis.

Fig. 1 – Micrographs illustrating staining for pro-BDNF in piglet DMNV (A–D), rhBDNF in DMNV (F–I), and TrkB in XII (K–N) in the: female control (A, F, K), female nicotine (B, G, L), male IHH (C, H, M), male nic+IHH (D, I, N) and negative stains (E (DMNV), J (DMNV), O (XII)). Positive neurons (black arrows) were distinguished from negative neurons (white arrows) by the brown colour staining in the cytoplasm (Scale bar=50 μm).