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BRAIN RESEARCH

Research Report

Changes in serotoninergic receptors 1A and 2A in the piglet brainstem after intermittent hypercapnic hypoxia (IHH) and nicotine

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ABSTRACT

We studied the effects of intermittent hypercapnic hypoxia (IHH) and/or nicotine on the immunoreactivity of serotoninergic (5-HT) receptors 1A and 2A in the piglet brainstem. These exposures were developed to mimic two common risk factors for Sudden Infant Death Syndrome (SIDS); prone sleeping (IHH) and cigarette smoke exposure (nicotine). Immunoreactivity for 5-HT_{1A}R and 5-HT_{2A}R were studied in four nuclei of the caudal medulla. Three exposure groups were compared to controls ($n=14$): IHH ($n=10$), nicotine ($n=14$), and nicotine + IHH ($n=14$). In control piglets, the immunoreactivity of 5-HT_{1A}R was highest in the hypoglossal nucleus (XII), followed by inferior olivary nucleus (ION), nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMNV), whereas for 5-HT_{2A}R, the immunoreactivity was highest in DMNV/NTS and then ION. Compared to controls, IHH reduced 5-HT_{1A}R immunoreactivity in all studied nuclei ($p<0.05$) but had no effect on 5-HT_{2A}R immunoreactivity. Nicotine reduced 5-HT_{1A}R immunoreactivity in the DMNV, ION and NTS ($p<0.001$), and reduced 5-HT_{2A}R immunoreactivity in DMNV/NTS ($p<0.05$). Nicotine + IHH reduced 5-HT_{1A}R in DMNV, ION and NTS ($p<0.001$) but had no effect on 5-HT_{2A}R immunoreactivity. Effects of nicotine on the DMNV were more significant in males compared to the females. These results show for the first time that IHH and/or nicotine can reduce 5-HT receptor immunoreactivity within functionally important nuclei of the piglet medulla. The findings support our hypothesis that 5-HT receptor abnormalities may be caused by postnatal exposures to clinically-relevant stimuli such as cigarette smoke exposure and/or prone sleeping.

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Abbreviations: 5-HT_{1A}R, serotoninergic receptor 1A; 5-HT_{2A}R, serotoninergic receptor 2A; DMNV, dorsal motor nucleus of the vagus; IHH, intermittent hypercapnic hypoxia; ION, inferior olivary nucleus; Nic + IHH, combined nicotine and IHH exposure; OD, optical density value; NTS, nucleus of solitary tract; SIDS, Sudden Infant Death Syndrome; XII, hypoglossal nucleus; % Positive, Average percentage of positively stained neurons; %Area of staining, Average percentage area of positive staining

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1. Introduction

The brainstem serotoninergic (5-HT) system is important for early brain growth and development, including the development of the central respiratory rhythm (reviewed in Richter et al., 2003). 5-HT receptors are classified into seven sub-families, 5-$HT_{1–7}$, which consist of a total of 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes (Baumgarten et al., 1997). Of the 14 receptor subtypes, the 5-HT_{1A} and 5-HT_{2A} receptors appear to be the main ones involved in brain development, despite their opposing functions in a variety of cellular and behavioural processes (Azmitia and Whitaker-Azmitia, 1997). In the central respiratory network, 5-HT_{1A}R efficiently reduces the excitability of respiratory neurons as indicated by the suppression of hypoxic activation (Richter et al., 1999), and decreased ventilatory responses to hypercapnia (Taylor et al., 2005); thus, 5-HT_{1A}R has inhibitory actions. 5-HT_{2A}R on the other hand, has an excitatory role and animal studies have shown that 5-HT_{2A}R receptor stimulation excites the respiratory motor system at the level of the pre-Botzinger complex resulting in increased gasping (Tryba et al., 2006), and increased frequency of two types of respiratory bursts representative of fictive eupneic activity and fictive sigh activity (Pena and Ramirez, 2002).

The cause of Sudden Infant Death Syndrome (SIDS) is still unknown; however, several risk factors have been identified. Currently, the two most important modifiable risk factors are cigarette smoking and prone sleeping. Serotoninergic studies within SIDS datasets have found decreased 5-HT receptor binding in the arcuate nucleus, raphe obscurus, paragigantocellularis lateralis, intermediate reticular zone, gigantocellularis nucleus (Panigraphy et al., 2000; Paterson et al., 2006), inferior olive (Panigraphy et al., 2000), hypoglossal nucleus and nucleus of the solitary tract (Paterson et al., 2006), and decreased 5-HT 1A and 2A receptor immunoreactivity in the dorsal motor nucleus of the vagus, nucleus of the solitary tract and ventrolateral medulla (Ozawa and Okado, 2002). In recent studies, polymorphisms in the promoter region (5HTTLPR) (Narita et al., 2001; Weese-Mayer et al., 2003a) and in intron 2 of SLC6A4, the gene responsible for the 5-HT transporter (5HTT) (Weese-Mayer et al., 2003b), were found in higher frequencies in SIDS cases compared to controls. These studies suggest that SIDS infants have a developmental and/or genetic disorder affecting the 5-HT system. However, studies in human postmortem tissue are limited with regards to their ability to evaluate the causes or consequences of these abnormalities.

Animal models are helpful for evaluating functional associations with histological findings. Using piglets, our laboratory has developed three models to mimic SIDS risk factors. These models include: 1—exposure to intermittent hypercapnic hypoxia (IHH), an exposure of increased carbon dioxide and decreased oxygen intake within a short period of time, which is aimed to mimic re-breathing episodes experienced by infants when sleeping in the prone position or with obstructive apneas (Waters and Tinworth, 2001); we chose this method of delivering the IHH stimulus rather than actual re-breathing or occlusion of the upper airways, because it provides a more controlled stimulus in terms of timing and gas levels achieved. 2—postnatal nicotine infusion to mimic postnatal nicotine intake via passive cigarette smoke exposure, or ingestion through breast milk, and 3—combined exposure of nicotine and IHH (Waters and Tinworth, 2001).

Nicotine is a component of cigarettes and was chosen for study because it is the most active agent and a neuroteratogen, which induces mitotic arrest, cell death, and loss of CNS cells, ultimately conceding synaptic function throughout the brain (Slotkin, 1998). Two previous studies have examined effects of nicotine on 5-HT_{1A}R affinity and mRNA expression in the brainstem, cerebral cortex and hippocampus of adolescent rats (Xu et al., 2002; Kenny et al., 2001). However, no study has looked at the effects of nicotine or of a hypoxic insult such as IHH on the serotoninergic system in the brainstem, during early postnatal development. We tested the hypothesis that postnatal exposures of nicotine and/or IHH decrease the immunoreactivity of 5-HT_{1A}R and 5-HT_{2A}R proteins in the developing piglet brainstem medulla and also examined for gender influences.

2. Results

2.1. *Piglet characteristics*

A total of 52 piglets were studied and separated into four groups; the control group, nicotine group, IHH group and nic+IHH group. The age at surgery for the nicotine groups was 0–2 days and age at death for all groups was 13–14 days. The body weight at surgery and at death, average weight gain and brain weight did not differ amongst groups. Detailed characteristics are provided in Table 1.

To confirm nicotine exposure, serum and urine cotinine levels were analysed in piglets exposed to nicotine. The cotinine levels at time of death were 20.3±2.5 ng/ml in serum (males: 19.8±5.0, females: 20.8±1.1), and 92.7±21.8 ng/ml in urine (males: 74.6±35.6, females: 113.7±20.1) respectively. For the nic+IHH group, the serum cotinine level was 25.5±3.8 ng/ml (males: 23.1±6.5, females: 51.3±22.6) and the urine cotinine level was 51.6±5.7 ng/ml (males: 51.9±8.1, females: 51.3±22.6). There were no statistical differences between genders or between nicotine vs. nic+IHH groups.

Table 1 – Piglet group characteristics

Characteristics	Control	Nicotine	IHH	Nicotine+IHH	*p* value
Number of piglets	14	14	10	14	–
Body weight at surgery (kg)	1.22±0.08	1.20±0.07	N/A	1.18±0.04	–
Average daily weight gain (g)	65.0±11.4	63.6±7.7	48.3±8.0	73.9±9.5	0.36
Body weight at death (kg)	2.0±0.17	1.9±0.12	1.7±0.10	2.0±0.13	0.47
Brain weight (g)	37.5±0.50	37.4±0.67	37.6±0.86	37.8±0.67	0.97