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Expression of kappa opioid receptors in developing rat brain – Implications for perinatal buprenorphine exposure

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ABSTRACT

Buprenorphine, a mu opioid receptor partial agonist and kappa opioid receptor (KOR) antagonist, is an emerging therapeutic agent for maternal opioid dependence in pregnancy and neonatal abstinence syndrome. However, the endogenous opioid system plays a critical role in modulating neurodevelopment and perinatal buprenorphine exposure may detrimentally influence this.

To identify aspects of neurodevelopment vulnerable to perinatal buprenorphine exposure, we defined KOR protein expression and its cellular associations in normal rat brain from embryonic day 16 to postnatal day 23 with double-labelling immunohistochemistry. KOR was expressed on neural stem and progenitor cells (NSPCs), choroid plexus epithelium, subpopulations of cortical neurones and oligodendrocytes, and NSPCs and subpopulations of neurones in postnatal hippocampus.

These distinct patterns of KOR expression suggest several pathways vulnerable to perinatal buprenorphine exposure, including proliferation, neurogenesis and neurotransmission. We thus suggest the cautious use of buprenorphine in both mothers and infants until its impact on neurodevelopment is better defined.

1. Introduction

Fetal and neonatal exposure to exogenous opioids is an increasing global problem. Examples of exposure include infants of mothers who use illicit or prescribed opioids during pregnancy and breastfeeding, as well as infants prescribed morphine or buprenorphine for treatment of neonatal abstinence syndrome (NAS) [1,2]. Among infants passively exposed to opioids in utero, 75%–90% have clinically significant withdrawal after birth, requiring pharmacological treatment for NAS [3,4]. In severe cases of NAS, collective pre- and postnatal exposure to opioids may even span months [5].

Buprenorphine, a mu opioid receptor (MOR) partial agonist and kappa opioid receptor (KOR) antagonist, shows increasing promise for the treatment of maternal opioid dependence in pregnancy and NAS. Its unique pharmacological properties and longer half-life allow extended dosage intervals and offer increased convenience, compliance and patient satisfaction over methadone, the current recommended pharmacological treatment for maternal opioid dependence, and morphine, the current recommended pharmacological treatment for NAS [6,7].

Current literature suggests that buprenorphine is potentially superior to methadone in reducing the incidence, severity and prognosis of NAS [5,8,9], is associated with a lower risk of preterm delivery, greater birth weight and larger head circumference [10] and has superior outcomes in both fetal and neonatal neurobehavioural scores [11,12]. A recent randomised controlled trial also found no significant difference in early childhood growth, cognitive development, language abilities, sensory pro-

Abbreviations: 3 V, third ventricle; BDNF, brain-derived neurotrophic factor; BT3, BetaTubulin-III; CA1, cornu ammonis area 1; CAq, cerebral aqueduct; CNS, central nervous system; CPE, choroid plexus epithelium; CSF, cerebrospinal fluid; DAPI, 4,6-diamidino-2-phenylindole; DG, dentate gyrus; Ex, embryonic day *x*; GCL, granular cell layer; GFAP, glial fibrillary acidic protein; KOR, kappa opioid receptor; LV, lateral ventricle; MBP, myelin basic protein; MOR, mu opioid receptor; NAS, neonatal abstinence syndrome; NDS, normal donkey serum; NeuN, neuronal nuclei; NMS, normal mouse serum; NRbS, normal rabbit serum; NSPCs, neural stem and progenitor cells; OR, opioid receptor; OSP, oligodendrocyte specific protein; Px, postnatal day *x*; PBS, phosphate-buffered saline; RGPs, radial glial progenitors; RMS, rostral migratory stream; RSG, retrosplenial granular cortex; SVZ, subventricular zone; TH, tyrosine hydroxylase; VZ, ventricular zone.

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cessing and temperament between children aged 0–36months exposed to buprenorphine or methadone in utero [13]. However, others have reported adverse outcomes such as prematurity, congenital malformations and neurodevelopmental deficits in visual-spatial ability, visual evoked potentials, motor skills, memory and attention [4,14–16]. Hence, it remains uncertain how perinatal buprenorphine exposure may affect normal fetal and neonatal neurodevelopment compared to current recommended therapies.

The endogenous opioid system plays a significant role in modulating neurodevelopment. The developing brain expresses the different classes of endogenous opioid ligands and opioid receptors (ORs) throughout embryonic and postnatal development in distinct regional and temporal patterns of distribution [17]. Exogenous opioid exposure in the perinatal period alters normal neurodevelopmental processes and is associated with long-term neurobehavioral changes such as impaired cognitive development, learning deficits and socio-behavioural issues [16,18,19]. However, with conflicting reports on OR expression and function within developing brain, the mechanisms underlying injuries associated with perinatal opioid exposure are not well understood.

2. Aim

We aimed to describe the distribution of KOR in normal developing rat brain and compare its expression to that of MOR. By defining their relationships to neural stem and progenitor cells (NSPCs), neurones and glia we aimed to identify the aspects of neurodevelopment which may be vulnerable to injury with perinatal buprenorphine exposure.

3. Materials and methods

Experimental work was undertaken with the approval of the University of New South Wales Animal Care and Ethics Committee in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines. Time-dated Wistar rats were supplied by the Animal Resources Centre (Perth, Australia) and sacrificed with intra-peritoneal injections of pentobarbitol sodium solution LethobarbTM (Virbac, Peakhurst, Australia). Embryonic and neonatal brains were harvested on embryonic day 16 (E16), E18, postnatal day 7 (P7), P15 and P23 (n = 2 at each time point), cardiac or immersion-fixed in ice-cold 4% paraformaldehyde, paraffin-embedded, sequentially sectioned from the ventral surface in a coronal orientation at 4 μm using a Leica semi-automated microtome (Leica, Nussloch, Germany) and mounted on Starfrost glass slides (Waldemar Knittel Glasbearbeitungs, Germany). Experimental methods used in our laboratory for embryonic and postnatal brain tissue collection have been previously described in detail [20].

Tissue sections were subjected to double-labelling immunohistochemistry with citrate wash antigen retrieval protocol as follows: de-paraffinised twice in Citrolene (POCD Scientific, Artamon, NSW, Australia), rehydrated through a graded ethanol series of 100%, 70% and 50% (POCD Scientific, Artarmon, NSW, Australia), then immersed for 5 min in Milli-Q water (Merck Millipore, Darmstadt, Germany). Antigens were retrieved by immersion in 100 °C sodium citrate, pH6 for 1 min before three 5 min phosphate-buffered saline (PBS) washes. Sections were then blocked for 20 min with 10% normal donkey serum (NDS, Sigma-Aldrich, D9663, St. Louis, MO, USA) diluted in PBS in a humidifier at room temperature. Primary antibodies, summarised in Table 1, were then added to each section before incubation for 1 h in a humidifier at room temperature. Negative controls used were 0.1% normal rabbit serum (NRbS, Sigma-Aldrich, R9133, St. Louis, MO, USA) or 0.1% normal mouse serum (NMS, Sigma-Aldrich, M5905, St. Louis, MO, USA) in PBS, in place of primary antibodies.

After incubation, sections were washed thrice with 5 min PBS washes and incubated for 20 min in a humidifier at room temperature with the secondary antibodies: Alexa Fluor Donkey anti-Rabbit 488 (A21206, Life Technologies, Mulgrave, Victoria, Australia) and Donkey anti-Mouse 594 (A21203, Life Technologies, Mulgrave, Victoria, Australia) in PBS, diluted at a concentration of 1:250 or 1:500. Following three 5 min PBS washes, sections were mounted with Fluoroshield with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, St. Louis, MO., USA) and cover-slipped. Slides were stored in the dark until examined.

Results were analysed using a Zeiss Axioplan2 microscope and Zeiss AxioCamMR digital camera, with Zeiss AxioVision software (Zeiss, Gottingen, Germany) allowing multichannel acquisition. Controls in which the primary antibody was omitted showed no specific staining. A minimum of 2 sections per antibody per animal were analysed. Images were prepared for presentation using CorelDraw software X5 (Corel Corporation 2010).

4. Results

KOR expression showed distinct patterns of regional and temporal variation. KOR expression was higher than MOR expression throughout embryonic and postnatal development.

4.1. Ventricular and subventricular zones

At E16, KOR was associated with ventricular zone (VZ) ependymal cells positive for nestin expression (nestin +) throughout the ventricular system. KOR+/nestin+ coexpression was especially prominent on the superior and medial aspects of the lateral ventricle (LV) ependyma and third ventricle (3 V) ependyma. Significant KOR+/nestin+ coexpression was also noted on NSPCs of the rostral migratory stream (RMS). KOR+/nestin+ coexpression was also noted on E18 olfactory bulb VZ.

Postnatally, KOR+/nestin+ coexpression continued to be observed on ventricular ependyma on the apical surface. With increasing maturity, KOR expression was found to be higher on 3 V and cerebral aqueduct (CAq) ependyma as compared to that of the LV. Nestin expression in VZ also declined with increasing gestation.

In subventricular zone (SVZ), KOR expression was lower than that in VZ. Coexpression with nestin was noted at P7. However, at P15 and P23, only KOR+/nestin- or KOR-/nestin+ cells were evident. No coexpression of KOR with beta-Tubulin 3 (BT3) or glial fibrillary acidic protein (GFAP) was noted in VZ or SVZ (Fig. 1).

4.2. Choroid plexus

KOR was expressed on choroid plexus (CP) of 3 V and LV from E16 to P23, and at a higher level than KOR expression on VZ. KOR expression on CP increased with advancing gestation. While nestin was also expressed on CP, this was associated with endothelium of developing blood vessels within the CP and not epithelial cells (Fig. 2).

4.3. Cortex

In embryonic cortex, there were only low levels of KOR expression evident. In postnatal cortex, while the majority of BT3+ developing neurones or NeuN+ neurones in postnatal cortex did not express KOR, discrete subpopulations of BT3+ or NeuN+ neurones did express KOR. This expression of KOR increased with gestation from P7 to P23; KOR expression at P7 was predominantly nuclear or cytoplasmic, while comparatively at P15 and P23, KOR was also expressed on cell membranes and occasional neuronal processes.