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Postnatal maturation of endogenous opioid systems within the periaqueductal grey and spinal dorsal horn of the rat

Charlie H.T. Kwok [a,*], Ian M. Devonshire [a], Andrew J. Bennett [b], Gareth J. Hathway [a]

[a] Laboratory of Developmental Nociception, Queens Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK
[b] FRAME Laboratory, Queens Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK

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ABSTRACT

Significant opioid-dependent changes occur during the fourth postnatal week in supraspinal sites (rostroventral medulla [RVM], periaqueductal grey [PAG]) that are involved in the descending control of spinal excitability via the dorsal horn (DH). Here we report developmentally regulated changes in the opioidergic signalling within the PAG and DH, which further increase our understanding of pain processing during early life. Microinjection of the μ-opioid receptor (MOR) agonist DAMGO (30 ng) into the PAG of Sprague-Dawley rats increased spinal excitability and lowered mechanical threshold to noxious stimuli in postnatal day (P)21 rats, but had inhibitory effects in adults and lacked efficacy in P10 pups. A tonic opioidergic tone within the PAG was revealed in adult rats by intra-PAG microinjection of CTOP (120 ng, MOR antagonist), which lowered mechanical thresholds and increased spinal reflex excitability. Spinal adminstration of DAMGO inhibited spinal excitability in all ages, yet the magnitude of this was greater in younger animals than in adults. The expression of MOR and related peptides were also investigated using TaqMan real-time polymerase chain reaction and immunohistochemistry. We found that pro-opiomelanocortin peaked at P21 in the ventral PAG, and MOR increased significantly in the DH as the animals aged. Enkephalin mRNA transcripts preceded the increase in enkephalin immunoreactive fibres in the superficial dorsal horn from P21 onwards. These results illustrate that profound differences in the endogenous opioidergic signalling system occur throughout postnatal development.

1. Introduction

Supraspinal modulation of spinal pain processing involves sensitive and precise orchestration of signalling between distinct anatomical regions, particularly in the periaqueductal grey (PAG) and nuclei of the rostral ventromedial medulla (RVM) [50]. These areas form part of a descending pain control axis, with the PAG integrating pain-related information from the forebrain and relaying appropriate neuromodulatory information to the dorsal horn (DH) of the spinal cord via pro- and antinociceptive descending pathways arising in the RVM [5,19,24].

Neonatal responses to noxious stimuli are exaggerated and often inappropriate [3,17,22,48]. The differences between mature and neonatal noxious processing are thought to be underpinned by increased excitation and decreased inhibition at the level of the DH [28]. Supraspinal control of spinal nociceptive reflexes is slow to develop over the postnatal period [29]. We have previously shown that although both descending facilitation and inhibition of spinal excitability can be evoked in adults when the RVM is electrically stimulated, in neonatal and juvenile rats, only descending facilitation can be evoked until at least postnatal day (P)28 [28].

It is known that pharmacological activation of μ-opioid receptors (MORs) within the adult descending pain modulatory pathway, particularly the PAG and RVM, results in potent analgesia [5,6,18–20,46,53,57]. Previously, we have shown that microinjection of the MOR agonist [D-Ala$_2$, N-MePhe$_4$, Gly-ol]enkephalin (DAMGO) into the RVM of lightly anaesthetised adult rats produces a dose-dependent decrease in spinal excitability, whereas the same dose of DAMGO in P21 rats produces reflex facilitation [29]. Additionally, we have demonstrated that blocking the central actions of endogenous opioid peptides with the potent opioid receptor antagonist naloxone between P21 and P28 prevents the normal development of descending RVM inhibitory control of spinal nociceptive reflexes [29].

These data indicate that the developmental transition from supraspinally mediated descending facilitation to inhibition of spinal excitability emanating from the RVM is controlled by opioidergic activity within the pain modulatory circuit during a critical period around P21. Furthermore, it suggests that there is a postnatal refinement in opioidergic neurotransmission within the central nervous system. Although existing evidence suggests that the

* Corresponding author. Tel.: +44 7717453080.
E-mail address: mbxck@nottingham.ac.uk (C.H.T. Kwok).

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switch in supraspinal control of spinal excitability during postnatal development may be driven by opioidergic activity within the pain modulatory circuit, much of this work has been done at the level of the RVM. A study of opioidergic activity in the PAG over postnatal development has been neglected.

In this study we demonstrate that significant refinement occurs within specific components of opioidergic systems of the PAG and DH, which has profound effects on the influences of these centres on pain processing.

2. Methods

2.1. Animals

All animal procedures were licensed by the UK Home Office and performed in accordance with the Animals (Scientific Procedures) Act 1986. Our experiments adhered to the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain. Postnatal day 3, day 14, and adult (240–260 g) Sprague-Dawley rats were purchased from Charles River Laboratories (Margate, UK). Pups were housed with their dams in individually ventilated cages in an in-house animal facility, weaned when they reached P21, and then group housed in same-sex cages. Free access to food and water was available throughout. Experiments were performed on P10, P21, and P40 (adult) rats, and different cohorts of rats were used in electrophysiological, immunohistochemical, and TaqMan real-time polymerase chain reaction (RT-PCR) studies. All procedures were performed during the animals' light cycle.

2.2. Surgery

PAG microinjection animals were anaesthetised with isoflurane (Baxter; Newbury, Berkshire, UK) and mounted on a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). The skull was exposed and bregma was located. Stereotaxic coordinates for the ventral PAG (vPAG) were calculated (both adult and P21: left-right [L-R] 0.5 mm; anterior-posterior −7.8 mm; dorsal-ventral [D-V] −6.0 mm; P10: L-R 0.5 mm; anterior-posterior −7.8 mm; DV −4.5 mm) and a 26-gauge 2.5-μL syringe (Hamilton, Reno, NV, USA) was inserted through a drilled hole in the skull. Drugs were injected over a 5-minute period, after which the syringe was removed and the wound was closed. Total volume of drug administered into the PAG was 1 μL; only one drug was administered per animal.

Laminectomy animals were anaesthetised and mounted on a stereotaxic frame. Laminectomy was performed to expose the L4-5 segments of the spinal cord. The dura mater was carefully removed, leaving the pia mater intact. This method allows the drug to be applied directly onto the spinal cord.

Drugs DAMGO (MOR-agonist, 30 ng; Tocris, Abingdon, Oxon, UK) and CTOP (MOR-antagonist, 100 ng; Tocris) were administered at doses determined from previously published studies in adult brainstem [28]. Saline was administered in separate sets of animals as vehicle control, and it was confirmed that saline either injected into the PAG or spinally applied had no significant effect on spinal reflex excitability. The experimenter was blinded to the drug administered. Sites of injection in the PAG were confirmed by examining the lesion tracts after electromyographic (EMG) recordings (Fig. 1). The brains were quickly dissected out and kept on dry ice, and were then coronally sectioned on a freezing microtome (Leica, Milton Keynes, Bucks, UK). The lesion sites were recorded. All DAMGO injection sites lay within the vPAG. Data from 2 adult rats following CTOP microinjection were excluded because the injection sites fell outside of the vPAG.

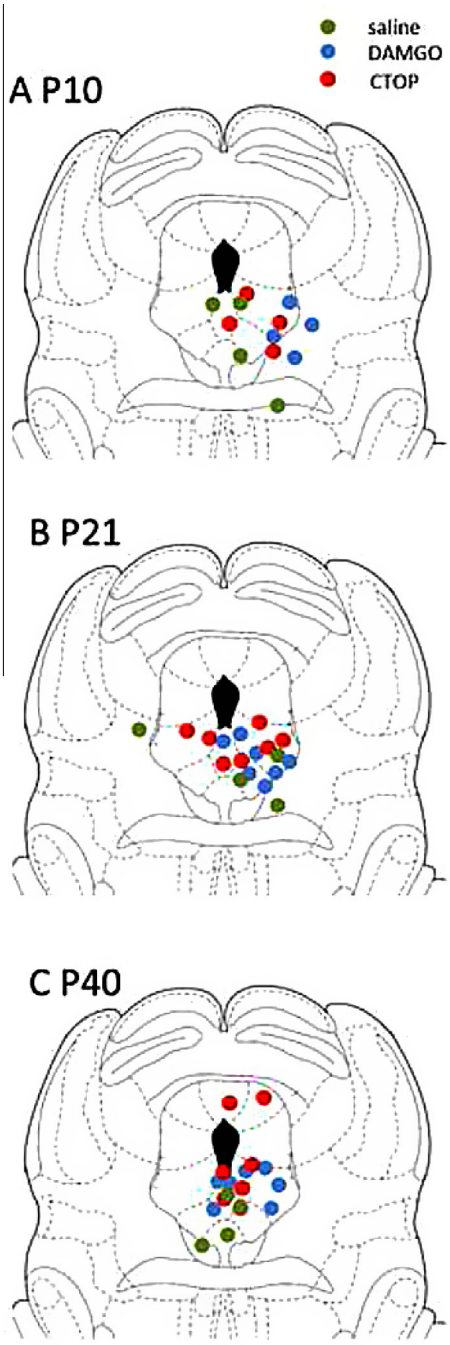

Fig. 1. Injection sites for saline, DAMGO (30 ng), and CTOP (120 ng) were identified histologically post electromyogram recording.

2.3. Electrophysiological recording

EMG recordings were performed as described previously [24]. Anaesthesia was initially induced at 5% isoflurane, which was then reduced to 3% for surgery. After surgery, isoflurane concentration was further reduced to 1.3% to keep the animals lightly anesthetised. Anaesthesia in P21 and adult rats were maintained with a surgically implanted endotracheal cannula, whereas in P10 rats it was maintained with a fitted nose cone. Animals were mounted onto the stereotaxic frame after tracheotomy. The fur overlying the biceps femoris muscle was trimmed and a bipolar concentric needle EMG recording electrode (comprising a modified 27-gauge hypodermic needle; Ainsworth, Coventry, UK) was inserted into