

μ and δ opioid receptor mRNA and protein expression in the cerebellum of the foetal, neonatal, and adult rat

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The three classical opioid receptors, μ , δ , and κ , are found in many regions of the rat brain where they act to modulate neurotransmission in the adult and regulate neurogenesis in the fetus and neonate. Based on radioligand binding and autoradiography, it is generally accepted that within the lobes of the rat cerebellum only δ opioid receptors are expressed. This is in contrast to the situation in humans and rabbits in which both μ and δ receptors are expressed in the cerebellum. Using frozen, paraformaldehyde-fixed cerebellar sections from foetal, neonatal, and adult Wistar rats, we investigated μ and δ opioid receptor protein distributions by immunohistochemistry, and opioid receptor mRNAs by fluorescent *in situ* hybridisation. Immunohistochemical staining using commercially available μ and δ antibodies followed standard procedures. For *in situ* hybridisation, riboprobes directly labeled with a fluorescent marker were used, thus, allowing comparative quantification of the message in brain tissue sections. cRNA probes complimentary to the 5' untranslated region of the mRNA were prepared. Targeting of this region rather than the coding region minimised cross hybridisation of cRNA probe between the closely related opioid receptor mRNAs that share significant regions of sequence similarity in their coding regions. Labeled cRNA probes were prepared from T7-tailed PCR products by *in vitro* transcription with T7 RNA polymerase and incorporation of Cy3-UTP or fluorescein-UTP into the reaction mixture. The results of this study showed that both μ and δ opioid receptor proteins and mRNAs were present in the adult and six day old neonatal rat cerebellum, specifically within Purkinje cells and in the granular layer. Expression of μ opioid receptor mRNA was also detected within cells of the molecular layer, but at lower levels than those seen within the Purkinje cells. Abundant expression of μ and δ opioid receptor mRNAs was also detected in the external germinal layer of the immature cerebellum of the foetal sixteen day post-conception rat, a finding that suggests a role for opioid receptors in neurogenesis of the developing cerebellum. Identification of both μ and δ opioid receptors within the developing cerebellum, and the known role of the cerebellum in coordinating multi-joint movements, supports the hypothesis that opioid receptors and their ligands affect development of coordinated movements in the neonate.