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Distribution of kisspeptin and its receptor in the canine hypothalamus


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Kisspeptins, a group of peptides encoded by the KiSS1 gene, play a key role in the hypothalamic-pituitary-gonadal (HPG) axis and are highly expressed in the hypothalamus of many species. Kisspeptin (KP) neurons directly stimulate GnRH neurons to secrete GnRH, which stimulates LH and FSH secretion by the gonadotropes in the pituitary gland. Negative and positive feedback on the HPG axis by gonadal steroids is mediated by down- or up-regulation through kiss1 mRNA in the hypothalamus [1]. Kisspeptin signaling is therefore an interesting target for future therapeutic intervention, for example estrus induction or prevention. It has been demonstrated that KP administration results in an increase of LH and FSH secretion in many species, including the dog [2]. However, there are no data about the distribution of KP or the KP receptor in the canine brain. The aim of the present study was to determine the distribution and localisation of KP and the KP receptor in the canine hypothalamus by immunocytochemistry. The hypothalamus was obtained from a Beagle bitch in anestrus and was transcardially fixed with a 4% buffered formalin solution. As primary antibodies, polyclonal rabbit antibody KiSS1R/GPR54 Antibody NLS1926 (Novus Biologicals, Littleton, CO), diluted 1:200 in PBS-T + BSA, and a polyclonal rabbit antibody Anti-Kisspeptin Antibody AB9754 (Merckmillipore, Billerica, MA) was used, diluted 1:1000 in PBS-T+BSA. As a secondary antibody a polyclonal goat anti-rabbit immunoglobulins/HPR P0448 (DAKO, Glostrup, Denmark) was used, diluted 1:500 in PBS-T + BSA. Normal rabbit serum (without the primary antibodies) was used as a negative control. After incubation with antibodies, sections were washed in PBS-T stained with DAB-substrate (SIGMAFAST, 3,3′-Diaminobenzidine tablets, D4168). Counterstaining was performed in 0.1% Cresyl Violet Acetate (Nissl staining). Both KP as the KP receptor were detected in the canine hypothalamus. The DAB precipitate was visible on neurons in the ventromedial area of the hypothalamus, located symmetrically on both sides of the third ventricle. The immunopositive cells were distributed homogeneously between the neurons in this region and the DAP precipitate was visible as punctae on the cell surface and in some neurons the staining was concentrated on the axon hillock. The negative controls showed no DAP precipitate. The area where KP and the KP receptor was detected in the canine hypothalamus corresponds to that of other species and is most likely to be the arcuate nucleus. As shown in other mammals, there are various nuclei in the hypothalamus in which KP and its receptor are expressed [2,3]. Gonadal steroids exert negative feedback on the HPG axis and it is hypothesized that they act on Kisspeptin neurons in the arcuate nucleus. In females, positive feedback (e.g. during the preovulatory LH surge) is regulated by another hypothalamic area, the anteroventral periventricular nucleus [2]. More research concerning the distribution of KP and its receptor in the canine brain is necessary to further understand the regulation of the HPG axis in the dog. For this purpose, the hypothalamus should be obtained from bitches during the various stages of the estrous cycle and double stained to detect the KP receptor and GnRH.