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RADIOLIGAND BINDING

SAIPHON SAWLON: IDENTIFICATION AND CHARACTERIZATION
OF DIFFERENT SUBTYPES OPIOID RECEPTORS IN BOVINE PINEALOCYTES.

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In this study an attempt has been made to identify and characterize opioid receptor subtypes in bovine pinealocyte membranes. By using the radioligand binding technique with the selective radioligands [^3H]-DAMGO, [^3H]-DPDPE, [^3H]-U69,593 and [^3H]-nociceptin for mu-, delta-, kappa- and ORL₁-opioid receptor subtypes, respectively. The saturation experiments and Scatchard analysis of specific opioid binding on bovine pinealocyte membranes provided maximal receptor density (B_{max}) and dissociation equilibrium constant (K_d) values. The B_{max} and K_d values obtained from the specific [^3H]-DAMGO binding was 6.34 ± 1.27 fmol/mg protein and 1.19 ± 0.44 nM, while the [^3H]-DPDPE binding values were 553.10 ± 24.24 fmol/mg protein and 1.28 ± 0.61 nM, respectively. On the other hand, the specific radioligands ([^3H]-U69,593 and [^3H]-nociceptin) binding of kappa and ORL₁ opioid receptors were undetectable in bovine pinealocyte membranes. Furthermore, the competitive experiments with opiate agonist and antagonist compounds confirmed the mu- and delta-opioid binding sites in bovine pinealocyte membranes. The rank order of affinities in [^3H]-DAMGO binding for the agents used was CTOP > met-enkephalin > DAMGO > leu-enkephalin > naloxone > naltrexone > DADLE > DPDPE, with the K_i values of 0.442 ± 0.094 , 0.569 ± 0.080 , 0.712 ± 0.125 , 1.250 ± 0.130 , 2.738 ± 0.245 , 5.923 ± 0.863 , 15.990 ± 2.010 and 29.040 ± 2.510 nM, respectively. In [^3H]-DPDPE binding, the rank order of affinities for the agents used was naltrindole > DADLE > ICI-174,846 > DSLET > met-enkephalin > DPDPE > leu-enkephalin > naloxone > naltrexone > DAMGO, with K_i values of 0.512 ± 0.082 , 0.698 ± 0.132 , 0.942 ± 0.157 , 1.064 ± 0.184 , 2.164 ± 0.263 , 6.632 ± 0.160 , 30.890 ± 2.915 , 47.990 ± 4.000 , 71.550 ± 18.380 and 436.30 ± 91.930 nM, respectively. The present results indicate that neither kappa nor ORL₁ receptors were found on the pinealocytes, as the majority of opioid receptors in the bovine pineal gland were delta type, with the minority being mu type, and that both were mostly located on the bovine pinealocyte membranes. Further studies on the molecular mechanisms regulating melatonin synthesis through opioid receptors are needed to reveal interaction among opioids and pineal gland functions.