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Development of a novel radioiodinated dermorphin analog for mu opioid receptor studies

Dermorphin is a naturally occurring opioid peptide that has been isolated from the skin of the frog Phylomedusa sauvagei. It has been characterized as having high affinity and selectivity for the mu opioid receptor. The goal of our study was to investigate novel analogs of dermorphin that could be radiolabeled and then evaluated as in vitro and in vivo probes for the mu opioid receptor. Our group synthesized DAPP, a novel peptide and dermorphin derivative with the amino acid sequence H-Dmt-D-Ala-Phe-Phe-NH2. It has been radiolabeled with iodine and used in various experiments to determine its receptor binding characteristics. Autoradiography studies were performed in order to define the profile of radioligand binding in mu, delta, and kappa opioid receptor types and to determine the degree of nonspecific binding and opioid receptor selectivity. Autoradiography is the detection of radioisotopes on X-ray film. For in vitro autoradiography, slide-mounted tissue sections of mouse brain were incubated with I-125 DAPP. The tissue specimen itself was the source of the radiation, and the emissions of the isotopes formed a visible image upon development. These studies indicated that I-125 DAPP exhibits high levels of binding in the frontal cortex, striatum, thalamus, and superior and inferior colliculi, and low levels in the cerebellum. The affinity of I-125 DAPP for mu opioid receptors was confirmed when labeling was not seen in the presence of a mu blocker (DAMGO); under the same conditions, I-125 DAPP was not prevented from binding by delta (DPDPE) or kappa (U69,593) blockers. Preliminary quantitative analysis of the distilled image by computerized densitometry suggests that we can achieve 80-90% specific binding with this compound. As a prelude to in vivo studies, we determined the distribution coefficient (log D) for I-125 DAPP at pH 7.4. This number is the ratio of the compound's concentration in the octanol phase to its concentration in the aqueous phase, and is an indication of the degree of lipophilicity of a compound. The log D for I-125 DAPP was determined as 2.99 ± 0.0117 (mean ± SD), using four consecutive measurements with a coefficient of variation (SD/mean x 100) of 0.39%. This relatively high value indicates a more lipophilic compound, suggesting that that I-125 DAPP will successfully cross biological membranes. An in vivo study was then conducted to determine the distribution of DAPP throughout the mouse body thirty minutes after injection. The highest levels of uptake were found in the small intestine, while penetration in the brain was minimal. These results suggest that I-125 DAPP is a valuable compound for in vitro studies, particularly autoradiography, but has limited utility for in vivo studies in normal mice. Nonetheless, I-125 DAPP may be suitable for in vivo targeting of certain cancers that over-express opioid receptors with respect to normal tissues.