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Sigma receptor binding in normal mouse lung membranes

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The brain and many peripheral tissues (e.g., heart, spleen and liver) express sigma receptors that have been characterized by radioligand binding studies in vitro. In vivo studies indicate that sigma receptors are expressed by normal lung, but the sites have not been studied in vitro. A screening assay we performed last year indicated the presence of sigma1 receptors in membranes from normal male CD1 mouse lung, and has sparked more detailed radioligand binding studies to characterize the properties of the binding site. Initial association data indicates substantial haloperidol-sensitive [3H]-(+)-pentazocine ([3H]-PTZ) specific binding to mouse lung membranes at 37 °C over a 4 hour incubation. However, a steady state is not reached by 8 hours, the longest time point tested. We then chose a 3 hour time point for lung saturation and competition binding assays based on known times for steady state of [3H]-PTZ in other tissues. Using [3H]-PTZ from 0.20 to 20.0 nM, we obtained a maximal receptor density (Bmax) of 847 +/- 63 (fmol/mg protein) and a ligand binding affinity (Kd) of 3.43 nM +/- 0.46 nM. Specific binding shows linear protein dependence between 75 and 300 ug per test tube. Although performed under non-steady state conditions, this “hot” saturation study indicates a high level of high affinity [3H]-PTZ recognition sites in normal mouse lung. Inhibition of [3H]-PTZ by “cold” PTZ gave an apparent affinity (Ki) of 5.31 nM +/- 0.19 nM, that is in good agreement with the measured Kd. Further studies will include using a panel of well characterized, non-radioactive ligands to establish a pharmacological profile for the putative sigma1 receptor sites labeled by [3H]-PTZ in normal mouse lung. Our characterization of sigma1 receptors on mammalian lung by radioligand binding should help provide a foundation for interpretation of in vivo studies.