In vivo receptor pharmacology studies with beta-adrenergic receptors in isolated perfused rat heart

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Kinetic techniques for in vivo receptor pharmacology studies using Positron Emission Tomography (PET) were investigated with the isolated perfused working rat heart and ligands that bind to β-adrenergic receptors. The high affinity β-adrenergic receptor antagonist ($-$)- ($\sp{125}$I) -iodocyanopindolol (ICYP) was the radiolabeled ligand used in all studies. Quantitative external detection of the radiolabel was used to measure the whole-heart kinetics of the labeled ligand. Compartmental modeling of the observed kinetics was performed to estimate the density of receptors $R\sb{\rm tot}$ and the strength of the binding interaction between the radiolabeled ligand and the receptors, reflected in the equilibrium dissociation constant K$\sb{\rm D}$.

Inhibition experiments were performed with the isolated rat heart and ICYP to measure the K$\sb{\rm D}$ values of unlabeled drugs that bind to β-adrenergic receptors. The five compounds studied and the corresponding K$\sb{\rm D}$ values measured in these inhibition experiments are: ($\pm$)-pindolol (K$\sb{\rm D}$ = 671 $\pm$ 123 pM), ($-$)-pindolol (K$\sb{\rm D}$ = 446 $\pm$ 53 pM), ($\pm$)-CGP12177 (K$\sb{\rm D}$ = 312 $\pm$ 45 pM), ($\pm$)-carazolol (K$\sb{\rm D}$ $\sim$ 37 $\pm$ 5 pM), and ($\pm$)-pindolol (K$\sb{\rm D}$ $\simeq$ 12 $\pm$ 3 pM).

The rationale behind a technique that uses two sequential bolus injections of radioligand to perform in vivo receptor pharmacology studies is explained. Experiments using a tracer injection of ICYP followed by a high mass injection that fills a significant fraction of the receptors yielded estimates of 77 $\pm$ 5 pmol/g dry for $R\sb{\rm tot}$ and (1.37 $\pm$ 0.02) $\times$ 10$\sp{-5}$ (pmol/g dry)$\sp{-1}$sec$\sp{-1}$ for $k\sbsp{\rm on}{*}$, the tissue association rate constant. The value of $R\sb{\rm tot}$ corresponds to $\sim$13 pmol/heart, with a typical rat heart weight of 1 g wet or 0.17 g dry. Variations of this two-injection protocol were also studied and yielded comparable parameter estimates.

Traditional in vitro experiments with ICYP and rat heart homogenates were performed to obtain values of $R\sb{\rm tot}$ and K$\sb{\rm D}$ which could be compared to kinetic measurements in the isolated heart. Saturation experiments yielded estimated values of 2.2 pmol/heart for $R\sb{\rm tot}$ and 9.2 pM for K$\sb{\rm D}$.

An approach to comparing binding parameters measured in vivo with kinetic techniques to those measured in vitro with equilibrium techniques was developed. This involved using the relative distribution volume (RDV) of ICYP in the heart, which was measured to be 864 $\pm$ 43 mL/g dry. This approach allowed the estimation of an aqueous K$\sb{\rm D}$ value of 4.5 $\pm$ 0.3 pM for ICYP from the kinetic parameter estimates. The RDV experiments also revealed a second class of binding sites for ICYP in the heart, with an $R\sb{\rm tot}$ of 1200 pmol/heart and a K$\sb{\rm D}$ of 17 nM.

The isolated rat heart offers a unique receptor system for pharmacological studies since the receptors remain intact in their original organ membranes. The developed techniques need to be further investigated to examine their ability to sensitively track changes in receptor density and affinity due to interventions such as myocardial ischemia and hypoxia.