Characteristics of (−)-[^{3}H]-dihydroalprenolol binding to β-adrenoceptors on rat lung membranes

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Much progress has been made in the identification and characteristics of the β-adrenoceptor by the use of binding studies with radiolabelled β-adrenoceptor antagonists (for review see Lefkowitz, Limbird, Mukherjee & Caron, 1976). In the present communication detailed binding characteristics of (−)-[^{3}H]-dihydroalprenolol ([^{3}H]-DHA) to rat lung membranes are described in an attempt to understand the nature and control of cell surface β-adrenoceptors in this tissue.

Lung parenchymal tissue (excluding major bronchi) was removed from male Wistar rats (120–150 g) and following homogenization and differential centrifugation the resulting membranes were used in the binding assay. The specific binding of [^{3}H]-DHA (binding that could be displaced by 200 μM (−)-isoprenaline) represented 85–95% of the total binding and was rapid ($T_{\frac{1}{2}}$ ass. = 4–5 min) and reversible ($T_{\frac{1}{2}}$ diss. = 10–15 min) at room temperature. The binding was also clearly saturable having a B max of 0.48 p mol/mg protein and a K_D of 0.5 nM. No co-operative interactions between the binding sites could be detected (slope of Hill plot = 1.1).

The data in Table 1 indicates that the binding of [^{3}H]-DHA was clearly stereoselective since (−)-propranolol was about 100 times more potent than (+)-propranolol in its ability to displace [^{3}H]-DHA from the membrane sites. Moreover, the relative potencies of a number of antagonists suggests that the receptors in rat lung are predominantly of the β_2 subtype. For example, H35/25 (1-(4-methylphenyl)-2-isopropylamine-propanol), a relatively specific β_2-antagonist, more potently inhibited [^{3}H]-DHA binding than (±)-atenolol and (±)-practolol (β_1-antagonists). However, detailed analysis of the displacement curves of (±)-atenolol and (±)-practolol using Hill plots suggests that a small proportion (20–25%) of the receptor sites may have β_1 characteristics. Identification of the cell type possessing β_2 and/or β_1 adrenoceptors remains to be established.

Table 1 Inhibition of [^{3}H]-DHA binding to rat lung membranes by β-adrenoceptor antagonists and agonists

Compound	*K_i(M)*
(−)-Propranolol	$4.1 \pm 0.7 \times 10^{-10}$
(−)-Timolol	$5.0 \pm 0.8 \times 10^{-10}$
(+)-Propranolol	$3.1 \pm 0.7 \times 10^{-8}$
(±) H35/25	$4.5 \pm 0.5 \times 10^{-7}$
(±)-Atenolol	$5.1 \pm 2.5 \times 10^{-6}$
(±)-Practolol	$1.6 \pm 0.5 \times 10^{-5}$
(−)-Isoprenaline	$2.8 \pm 1.0 \times 10^{-7}$
(−)-Adrenaline	$0.9 \pm 1.0 \times 10^{-6}$
(±)-Salbutamol	$1.2 \pm 0.1 \times 10^{-6}$
(−)-Noradrenaline	$4.9 \pm 0.5 \times 10^{-6}$

Compounds were incubated at various concentrations with lung membranes (100–200 μg protein) and [^{3}H]-DHA (0.7–1.5 nM) at room temperature for 30 min in a final volume of 250 μl. Samples were then rapidly vacuum filtered over glass fibre filters and the membrane-bound radioactivity retained by the filters was determined by liquid scintillation counting. IC_{50} values were calculated from the percentage inhibition of [^{3}H]-DHA binding using Hill plots. The K_i was then calculated from the equation $K_i = IC_{50}(1 + [S]/K_D)$ where [S] is the concentration of [^{3}H]-DHA used in the assay and $K_D = 0.5$ nM. All results are expressed as mean ± s.e. from a minimum of 3 experiments on different groups of rat lung membranes.

Reference

LEFKOWITZ, R.J., LIMBIRD, L.E., MUKHERJEE, C. & CARON, M.C. (1976). The β-adrenergic receptor and adenylate cyclase. *Biochim. Biophys. Acta*, **457**, 1–39.