## Characterization of ß-adrenoceptors of the $BC_3H1$ nonfusing muscle cell line

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The clonal cell line $BC_3H1$ is a non fusing muscle cell line isolated by Schubert, Harris, Devine & Heinemann (1974). We have shown previously that different catecholamines increase the transmembrane $^{86}Rb$ efflux by the stimulation of α- and β-adrenoceptors (Mauger, Moura & Worcel, 1978). In order to better characterize the β-adrenoceptors, we have compared the effect of some β-adrenoceptor agonists and antagonists on the $^{86}Rb$ efflux from $BC_3H1$ cells, with the binding properties of the radiolabelled β-adrenoceptor antagonist [$^3$H]-dihydroalprenolol ([$^3$H]-DHA). The experiments were performed on cells in stationary phase of growth. The technique used for the efflux experiments has been described previously (Mauger, Moura & Worcel, 1978).

For the [$^3$H]-DHA binding studies, a crude membrane fraction was prepared from cells grown in 90 mm diameter Petri dishes.

The action of agonists and antagonists on the $^{86}Rb$ efflux was studied in the presence of phentolamine ($10^{-6}$M) in order to block α-adrenoceptors. Under these conditions the dose-response curves obtained result from the stimulation of β-adrenoceptors. The $EC_{50}$ obtained were: isoprenaline ($1.6 \times 10^{-8}$M); adrenaline ($1.9 \times 10^{-8}$M) and noradrenaline ($1.1 \times 10^{-6}$M). Alprenolol and propranolol act as competitive antagonists of adrenaline action with $pA_2$ values of 8.7 and 8.5 respectively.

Specific binding of [$^3$H]-DHA on $BC_3H1$ cell membranes, defined as the difference between the total binding and the non-specific binding measured in the presence of isoprenaline ($10^{-5}$M), reached equilibrium by 10 min at 30°C. This binding was reversed by a large excess of isoprenaline with a $T_{\frac{1}{2}}$ of 5 min. The Scatchard analysis performed on binding curves, obtained in equilibrium conditions, shows a single class of sites for [$^3$H]-DHA, with a $K_D$ of 0.6 nM and a maximal binding of 60 fmoles/mg of protein. We tested the ability of several adrenoceptor agonists and antagonists to displace [$^3$H]-DHA from its binding sites. The $K_D$ value for each ligand was calculated from the $EC_{50}$ by the method of Cheng & Prusoff (1973). The $K_D$ observed were: isoprenaline ($4.7 \times 10^{-8}$M); adrenaline ($3 \times 10^{-7}$M); noradrenaline ($2.8 \times 10^{-6}$M); alprenolol ($2 \times 10^{-9}$M); propranolol ($7 \times 10^{-9}$M); butoxamine ($1 \times 10^{-6}$M) and practolol ($1.9 \times 10^{-5}$M).

The results of the present experiments confirm that the β-adrenoceptors of the $BC_3H1$ line corresponds to the $\beta_2$ type described by Lands, Arnold, McAuliff, Luduena & Brown (1967), as suggested in our previous paper (Mauger, Moura & Worcel, 1978).

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## ß-Adrenoceptor binding sites in rat spleen: pharmacological characteristics and effects of chemical sympathectomy

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The development of receptor binding studies using radiolabelled ligands has been of particular use in the identification and characterization of β-adrenoceptors in several tissues (Wolfe, Harden & Molinoff, 1977). Recent studies using this approach have established that β-adrenoceptor binding sites are not homogenous and that in lung and cerebral tissue at least, $\beta_1$ and $\beta_2$ receptor subtypes co-exist in varying proportions (Barnett, Rugg & Nahorski, 1978; Nahorski, 1978). In the present communication we have examined the characteristics of β-adrenoceptors in the spleen, since experiments in intact preparations have suggested that a proportion of the receptors may be presynaptic in this tissue and may regulate noradrenaline release by positive feedback (Langer, 1977).

Spleens were removed from male Wistar rats (120–150 g) and following homogenization and differential centrifugation, washed membranes were used in the binding assay. The specific binding of the ligand (–) [$^3$H]-dihydroalprenolol ([$^3$H]-DHA) (binding displaced by 200 μM isoprenaline) represented 60–70% of the total binding and was clearly saturable. Scatchard analysis revealed that the