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 (1.6×10^{-8}M); adrenaline (1.9×10^{-8}M) and noradrenaline (1.1×10^{-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 (4.7×10^{-8}M); adrenaline (3×10^{-7}M); noradrenaline (2.8×10^{-6}M); alprenolol (2×10^{-9}M); propranolol (7×10^{-9}M); butoxamine (1×10^{-6}M) and practolol (1.9×10^{-5}M).

The results of the present experiments confirm that the β-adrenoceptors of the BC_3H1 line corresponds to the β_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, β_1 and β_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

total number of binding sites (Bmax) was 52 ± 17 fmoles × mg protein^{-1}, and the affinity of [^{3}H]-DHA for the splenic binding sites was 0.69 ± 0.17 nM. Pharmacological characterization of the labelled sites demonstrated that the binding of [^{3}H]-DHA could be displaced by a number of β-adrenergic agonists and antagonists with relative affinities that match their pharmacological potency at the β-adrenoceptor.

However, whereas (−)-timolol, a non-selective β-adrenoceptor antagonist, competed for a single population of high affinity sites ($K_i = 0.69$ nM), agents that demonstrate high selectivity for β_1 or β_2 adrenoceptors ((±)-practolol, (±)-atenolol (β_1), or procaterol (β_2)) produced flattened displacement curves. Analysis of the competition curves suggests that 30–35% of the labelled sites have high affinity for β_1 drugs, whereas the remainder can be displaced with high affinity by β_2 agents.

In an attempt to determine whether β_1 or β_2 receptors were associated with presynaptic sites, rats were treated with 6-hydroxydopamine (2 × 50 mg/kg + 2 × 70 mg/kg i.v.) over two weeks to destroy noradrenergic nerve terminals in the spleen. This treatment produced a severe depletion (control 1130 ± 84, treated 271 ± 31 pmole × gm^{-1}) of splenic noradrenaline that was maintained for at least seven days after the last injection. However, membranes prepared from treated rats possessed β-adrenoceptor binding sites with properties (number of sites, affinity for [^{3}H]-DHA, proportions of β_1 and β_2 receptor sites) that were virtually identical to controls.

Although it was not possible to identify presynaptic β-adrenoceptors in rat spleen, it appears possible that these sites represent a very small proportion of the total β-adrenoceptor population. However, the present experiments do suggest that since the properties of [^{3}H]-DHA binding sites were unaltered when presynaptic nerve terminals were destroyed by 6-hydroxydopamine, both β_1 and β_2 adrenoceptor subtype co-exist postsynaptically in this tissue.

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Effect of diazepam and its metabolites on the binding of L-tryptophan to human serum albumen

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Benzodiazepines compete with L-tryptophan for the binding site on the albumen molecule. In the rate, chlordiazepoxide (20 mg/kg i.p.) produces an increase in free tryptophan in plasma with an increase in the concentration of tryptophan and 5-hydroxytryptamine in the brain (Bourgoin, Héry, Ternaux & Hamon, 1975). We have studied the effects of diazepam and its metabolites on the affinity constant of L-tryptophan for purified human serum albumen (HSA), and the effect of diazepam on tryptophan binding in normal human serum using a drug concentration (35 μM) which is somewhat higher than that which would be expected in the therapeutic situation (Bond, Hailey & Lader, 1977).

In the studies with purified HSA, solutions were prepared containing L-([^{14}C]-methylene) tryptophan (0.17 μCi/ml:3 μM), L-tryptophan (six concentrations between 12 and 200 μM), HSA (approximately 75 μM, Sigma – essentially fatty acid free), PEG 200 (0.5%) and its benzodiazepine (50 μM) where appropriate, in tris buffer (50 μM: 7.4). These were left for 1 h at room temperature, and the free L-tryptophan separated by ultrafiltration through Amicon CF 25 cones (5 min at 900 g). Radioactivity in the mixture and ultrafiltrate was measured by liquid scintillation counting. Total L-tryptophan concentrations were determined using an amino acid analyser, and the albumen concentration by the bromo-cresol green assay. In the studies with human serum, blood was taken at 0900 h from five healthy male subjects. Portions of the pooled serum were prepared containing L-([^{14}C]-methylene) tryptophan (0.033 μCi/ml:0.6 μM), and diazepam (35 μM) or saline (control), and pH was maintained under an atmosphere of 95% air and 5% carbon dioxide. The free and total tryptophan concentrations were determined as before.

Linear regressions were fitted to the data for the Scatchard plots ($P<0.01$) and gave intercepts on the abscissae close to unity for control and all drugs (0.93–1.11). The mean association constant of L-tryptophan for HSA (17.7 × 10^{3}M^{-1}) was changed by diazepam ($K = 8.6 \times 10^3 M^{-1}$: $P<0.01$), temazepam ($K = 8.8 \times 10^3 M^{-1}$: $P<0.05$), oxazepam ($K = 9.4 \times 10^3 M^{-1}$: $P<0.05$), and nordiazepam ($K = 11.9 \times 10^3 M^{-1}$: $P<0.01$), but there were no differences between the association constants of in-