Short term regulation of \( \beta \)-adrenoceptors in peripheral blood mononuclear cells after sympathetic activation has been previously documented in normal individuals but changes after a central reduction in sympathetic activity are not known. We have studied \( \beta \)-adrenoceptor number and affinity on peripheral blood mononuclear cells in normal subjects, before and after intravenous clonidine, an \( \alpha_2 \)-adrenoceptor agonist which lowers blood pressure predominantly by reducing central nervous system sympathetic outflow. After clonidine there was a decrease in plasma levels of noradrenaline and adrenaline, and an increase in growth hormone. There was up-regulation of \( \beta \)-adrenergic receptors on peripheral blood mononuclear cells 30 and 60 min after clonidine which was related to the fall in blood pressure, noradrenaline and adrenaline levels and to the increase in growth hormone levels. The affinity of the receptors was decreased. Return to baseline values was observed after 2 h. Intracellular production of cAMP after isoproterenol stimulation demonstrated that the up-regulation was not functional. Our studies indicate short term up-regulation of \( \beta \)-adrenoceptors in peripheral blood mononuclear cells after clonidine. These observations after a reduction in sympathetic activity may be of importance if they mirror the pattern of redistribution of adrenoceptors, which are present in a wide range of tissues.

**Key words:** \( \beta \)-adrenoceptors, Healthy controls, Clonidine

**Introduction**

Adrenergic receptors of blood cells in humans have been used extensively in *in vitro* studies as a model for adrenoceptors in other parts of the body. In particular, lymphocytes have been frequently used to evaluate \( \beta_2 \)-adrenergic receptor function. There is a correlation between the number of \( \beta_2 \)-adrenoceptors on lymphocytes and heart and lung,\(^1,2\) although it is not clear to what extent changes in peripheral blood mononuclear cells (PBMC) adrenoceptors mirror adrenoceptor changes in other tissues. These studies however, have increased our understanding of the mechanisms involved in adrenergic receptor regulation.\(^3-5\)

The short and long term effect of catecholamines and various drugs *in vitro* and *in vivo* upon adrenergic receptors on blood cells have been extensively investigated. Reduced \( \beta \)-adrenergic receptor numbers have been reported during chronic \( \beta \)-adrenoceptor agonist treatment.\(^6\) Conversely, increased receptor sites on lymphocytes have been observed during long term administration of \( \beta \)-blockers.\(^7\) During infusion of catecholamines the findings support an early up-regulation\(^8,9\) and a later down-regulation\(^9,10\) of \( \beta_2 \)-adrenergic receptors on PBMC. Exercise can increase \( \beta_2 \)-adrenoceptor number and function on lymphocytes.\(^11,12\) These findings were attributed to sympatho-adrenal activation. While some knowledge of the effects of sympatho-adrenal activation or sympathomimetic action of drugs upon the short term regulation of \( \beta \)-adrenoceptors has been gained through these studies, little is known about the reverse, sympatho-adrenal inactivation. One approach has been the study of patients with autonomic failure (AF), and in these chronic models of sympathetic denervation an increased number of \( \beta \)-adrenergic receptors has been reported.\(^13\) In the present study we have used a pharmacological approach to evaluate the effects of a short term reduction in central sympathetic outflow caused by the \( \alpha_2 \)-adrenoceptor agonist clonidine, upon \( \beta \)-adrenoceptor number and function of PBMC in normal subjects.

**Subjects and Methods**

We studied 15 healthy normal subjects (age: 41 ± 11 years; mean age: 23–55 years). None was on medication and screening tests excluded autonomic dysfunction. All were studied after an overnight fast in a temperature controlled room (22°C) after 30 min supine rest, to allow familiarization with equipment. Measurements were then made before and 15, 30, 45 and 60 min after clonidine (2 \( \mu g/kg \) infused i.v. over 10 min). Blood pressure (BP) and heart rate (HR) were assessed by an automated sphygmomanometer (Sentron).
Blood samples for determination of plasma levels of noradrenaline (NA), adrenaline (A), growth hormone (GH) and \( \beta \)-adrenergic receptor studies were drawn from a cannula (Abbocath-T in a forearm vein, 18 G) into heparinized 10 ml Vacutainer tubes.

**Plasma noradrenaline, adrenaline, dopamine and growth hormone measurements.** Plasma from heparinized blood samples was aliquoted and stored at \(-20^\circ\)C. Plasma samples were thawed and noradrenaline (NA) and adrenaline (A) and dopamine (DA) levels were measured by high performance liquid chromatography (HPLC).14 The intra-assay and inter-assay coefficients of variation were 10%. Growth hormone was measured using \(^{125}\)I-labelled growth hormone radioimmunoassay kit (NETRIA); the results were calculated and levels expressed in mU/l. The intra-assay and inter-assay coefficients of variation were 8.5% and 9.6% respectively.

**Beta adrenergic receptor assay.** PBMC were isolated according to the method of Boyum.15 The mononuclear layer was washed twice in PBS. Viability was 95% as assessed by eosin (2% w/v) exclusion. PBMC were resuspended at a concentration of \(5 \times 10^6\) cells/ml. The ligand binding assay was carried out in triplicate in round-bottom 96 well plates using the \( \beta \)-adrenergic antagonist \(^{125}\)I Iodocyanopindolol (CYP) (Amersham, UK), sp. act. 2,000 cpm/mmol. Total binding was determined by incubating \(5 \times 10^5\) cells in 200 \(\mu\)l volume with seven concentrations (range 25 to 200 \(\mu\)M) \(^{125}\)I CYP for 90 min at \(30^\circ\)C. Non-specific binding was determined over the same concentration range of \(^{125}\)I CYP in the presence of 1 \(\mu\)M dl-propranolol (Sigma, UK) which displays the maximum of the total \(^{125}\)I CYP binding on the top concentration. After incubation the cells were harvested using a Titerlek cell harvester on filter mats (SKATRON, UK). The mats were dried and the discs counted in a LKB mini-gamma counter for 60 s. Binding kinetics were determined by Scatchard analysis using an EBDA/LIGAND computer program.16

**Measurement of intracellular cAMP.** The PBMC were washed once in 50 mM Tris–HCl buffer, pH 7.4, containing 0.9% NaCl, 4 mM EDTA and 8 mM theophylline. Both theophylline and EDTA strongly inhibit phosphodiesterase activity, and were used in the assay to prevent breakdown of cAMP. The cells (\(5 \times 10^5\) per incubation) were suspended in 0.2 ml of the same buffer containing \(10^{-4}\) M (–)-isoproterenol, the concentration producing maximal cAMP (dose–response curve data not shown). Cells were incubated in the presence and absence of (–)-isoproterenol for the determination of basal levels of intracellular cAMP. The cell suspensions were incubated for 15 min at \(37^\circ\)C in a shaking water bath. The reaction was terminated by addition of 0.3 ml ice-cold distilled \(\mathrm{H}_2\mathrm{O}\) and vortexing for 5–10 s. The suspension was rapidly frozen in liquid nitrogen. All cells were disrupted by this treatment, as judged by light microscopy. The amount of cAMP in the lysates was determined using a commercial kit (cyclic AMP \(^{125}\)I assay system single range RPA.508, Amersham, UK.)

The intra-assay and inter-assay coefficients of variation were 6.8% and 10% respectively. Results were expressed as fmol cAMP/10\(^6\) cells.

**The effect of clonidine on \( \beta \)-receptor density in cultured PBMC.** PBMC were washed in basal Medium EAGLE (Gibco) under sterile conditions. The cells were cultured in Dutch modified RPMI-1640 (Gibco) supplemented with 10% foetal calf serum (FCS), 100 \(\mu\)IU/ml penicillin, 100 \(\mu\)g/ml streptomycin, in the presence and absence of 4 \(\mu\)M clonidine; 4 \(\mu\)M being the peak plasma concentration of the drug 90 min after oral administration of 300 \(\mu\)g.17

**Statistical analysis.** Data are presented as mean ± SEM. The Student’s \( t \)-test for paired samples was used. Statistical significance was accepted at 95% confidence level (\(p < 0.05\)).

**Results**

The major findings are presented in Table 1. A statistically significant fall in BP (mean, systolic and diastolic; mean arterial blood pressure was calculated by dividing systolic blood pressure plus two times the diastolic blood pressure by three) was observed after clonidine at 15, 30, 45, and 60 min. Heart rate (HR) did not change. Mean plasma levels of NA fell by 50% in 13 out of 15 subjects but high levels in two individuals affected the SEM and the fall did not reach significance. Levels of A fell significantly. Plasma levels of dopamine (DA) remained unchanged. Significant increase in densities of \( \beta \)-adrenoceptors on PBMC was observed at 30 and 60 min. In seven subjects a time course assessment demonstrated that \( \beta \)-adrenoceptors returned to baseline values 2 h after the clonidine (Fig. 1). The receptor affinity also changed with a substantial decrease at 30 and 60 min. The receptor binding assays were saturable and competitive. The receptor affinity was expressed as \( \mathrm{Kd} \) in \(\mu\)M and the receptor density with \(B_{\max} \) expressed as sites/cell and normal range of 1344 ± 140.13 The binding versus free linear regression analysis diagrams are presented in Fig. 2. No changes in basal and stimulated intracellular production of cAMP were observed on PBMC at 0, 30 and 65 min after clonidine infusion (Fig. 3). In vitro culture of PBMC from four normal subjects in the presence of 4 \(\mu\)M