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Human β-Adrenoceptors: Relation of Myocardial and Lymphocyte β-Adrenoceptor Density

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In human right atria obtained from 21 patients during open-heart surgery, β-adrenoceptor density [assessed by iodine-125–labeled (−)-cyanopindolol binding] and responsiveness (positive inotropic responses to isoprenaline) were linearly related to the β-adrenoceptor density in the corresponding circulating lymphocytes. This direct relation of human myocardial and lymphocyte β-adrenoceptor alterations, therefore, makes it possible to monitor drug- or disease-induced β-adrenoceptor changes in tissues not easily accessible in humans.

THE DEVELOPMENT OF RADIOLIGAND binding techniques for direct identification of receptors has advanced our knowledge of the molecular pharmacology of β-adrenoceptors (*1, 2*). The number of β-adrenoceptors on cells is dynamically regulated by a variety of drugs, hormones, and physiological and pathological conditions (*3*). Circulating lymphocytes containing a homogeneous population of β_2-adrenoceptors (*4*) excitatorily coupled to adenylate cyclase (*5*) are used to study such alterations of β-adrenoceptor function (*6*) in humans. However, despite similar in vitro properties of β-adrenoceptors (as determined by radioligand binding studies) in blood cells and various tissues, the relation of β-adrenoceptor changes measured in circulating lymphocytes to changes potentially occurring in solid tissues has not been established. We report here that, in human subjects, the density of β-adrenoceptors in circulating lymphocytes is significantly related to the density and responsiveness of β-adrenoceptors in right atrial appendages from the same subjects.

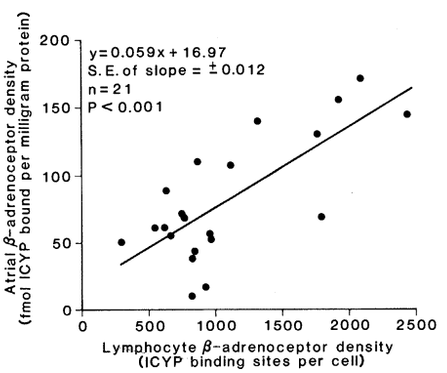

Fig. 1. Relation of β-adrenoceptor density (determined by Scatchard analysis of specific ICYP binding at six to eight concentrations ranging from 10 to 150 p*M*) on membranes from human right atria and on intact cells of the corresponding lymphocytes.

Human right atrial appendages were obtained from 21 patients [18 males and 3 females 54.6 ± 1.3 years old (mean ± standard error; range, 41 to 64 years)] undergoing elective coronary artery bypass grafting. No patient suffered from acute myocardial failure and none had been treated with catecholamines for at least 3 weeks before surgery. However, the patients had received nitrates (n = 19), calcium antagonists (n = 16), and occasionally β-blockers (metoprolol, 50 mg twice daily, two patients and 100 mg twice daily, one patient; and atenolol, 50 mg once daily, one patient and 100 mg once daily, one patient). Preoperative medication consisted of flunitrazepam and atropine; the operation was done under balanced anesthesia with fentanyl, isoflurane, etomidate, and flunitrazepam. In some cases N_2O was added. Pancuronium was used as a muscle relaxant.

In all patients the right atrial appendages were removed under normothermic conditions before cardiopulmonary bypass. Immediately after removal, all specimens were placed in a sealed vial with Krebs-Henseleit solution (119 m*M* NaCl, 2.5 m*M* $CaCl_2$, 4.8 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 24.9 m*M* $NaHCO_3$, 10.0 m*M* glucose, and 0.057 m*M* ascorbic acid) aerated by "carbogen" (95 percent O_2 and 5 percent CO_2) at room temperature and transported immediately to the laboratory. The preparation of the tissues was begun within 5 to 15 minutes of surgical removal. The atrial appendages were first divided in two parts: one was used to determine β-adrenoceptor density; the other was dissected to yield trabecular strips (diameter, 1 mm or less) 4 to 5 mm long without endocardial damage for determination of mechanical responses to isoprenaline.

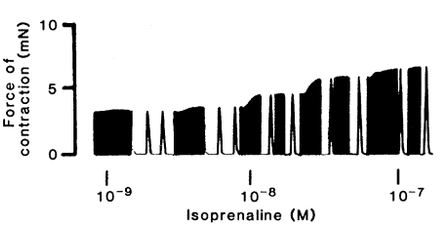

Fig. 2. Positive inotropic effect of isoprenaline on the isolated electrically driven muscle strip of human right atria. The concentration of isoprenaline was increased in steps of 0.5 log units.

The preparations were mounted in a 50-ml organ bath containing Krebs-Henseleit

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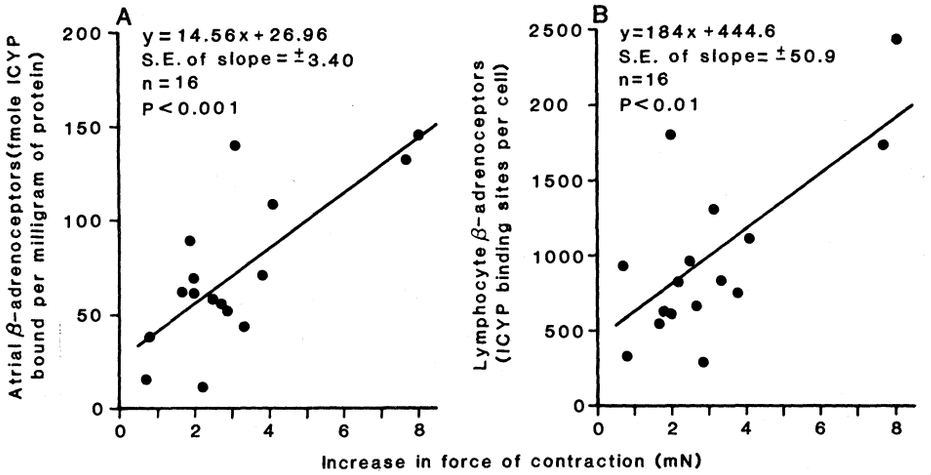

Fig. 3. Relation between the maximum increase in force of contraction of the isolated human right atria induced by saturating concentrations of isoprenaline and (A) the β-adrenoceptor density in the corresponding atria and (B) circulating lymphocytes.

solution equilibrated with carbogen at a temperature of 37°C; they were electrically stimulated at 1.0 Hz by square-wave impulses with a duration of 5 msec and a voltage 20 percent above threshold. The developed tension of the atria maintained under a resting tension of 4.9 mN was recorded with a strain gauge on a Hellige recorder. All preparations were incubated once for 2 hours with 5 μM phenoxybenzamine to prevent tissue uptake of isoprenaline. Isoprenaline produced positive inotropic effects in 16 of the 21 atria. Cumulative dose-response curves for the positive inotropic effect of isoprenaline were determined (*7*). The density of β-adrenoceptors was assessed in atria on membrane preparations and in the corresponding lymphocytes on intact cells by binding with the selective β-adrenoceptor radioligand (−)-[^{125}I]cyanopindolol (ICYP) (*8, 9*).

The mean number of β-adrenoceptors in right atrial membranes obtained from the 21 patients was 81.06 ± 10.1 (range, 10.3 to 172) fmol of ICYP bound per milligram of protein, which agrees with recently reported data from our laboratory [86.4 ± 7.4 fmol/mg, n = 4 patients (*8*)] as well as from Stiles *et al.* [83.8 ± 13.8 fmol/mg, n = 3 patients (*10*)]. The binding affinity (K_d) value for ICYP was 20.8 ± 3.3 pM. In the corresponding lymphocytes obtained from the same patients, the mean β_2-adrenoceptor density was 1087 ± 125 (range, 288 to 2442) ICYP binding sites per cell (K_d for ICYP, 19.8 ± 2.7 pM). In both tissues there was no significant linear relation between β-adrenoceptor density and age, although β-adrenoceptors showed a slight tendency to increase with age (*11*). Examination of the data for all 21 subjects revealed a highly significant linear relation between the density of β-adrenoceptors in atrial membranes and in the corresponding lymphocytes (Fig. 1).

The maximum increase in the force of contraction evoked by isoprenaline on the isolated electrically driven human right atria was 3.12 ± 0.56 mN (n = 16); the pD_2 value for isoprenaline (negative logarithm of the molar concentration of isoprenaline causing half-maximum effects) was 7.74 ± 0.71 (n = 16) (Fig. 2). There were highly significant linear relations between the maximum contractile responses of the atria to isoprenaline and the corresponding atrial (Fig. 3A) and lymphocyte β-adrenoceptor densities (Fig. 3B).

Responses of β-adrenoceptors to catecholamines are altered in a variety of circumstances, including disease states and chronic exposure to hormones or drugs (*2, 3*). Circulating lymphocytes having a homogeneous population of β_2-adrenoceptors are generally used to study such β-adrenoceptor alterations (*6*). Our results show that changes in β-adrenoceptors measured in circulating lymphocytes mirror changes in the density and functional responsiveness of β-adrenoceptors in solid tissues, at least in the human heart. Accordingly, it may be possible to determine the density and functional responsiveness of β-adrenoceptors in circulating lymphocytes in order to monitor drug-induced β-adrenoceptor changes (for example, β-blockers in the treatment of hypertension and β_2-agonists in the therapy of asthma), which might help to improve the effectiveness of drug treatment.

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