Alterations in Cardiac β-Adrenoceptors During the Development of Rat Experimental Autoimmune Myocarditis

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Summary. In this study, we investigated changes in cardiac β-adrenoceptor densities (Bmax) and affinities (dissociation constant: Kd) by Scatchard analysis using the radioligand binding assay method during the development of rat experimental autoimmune myocarditis (EAM). Rats at 11 weeks of age were subcutaneously injected in both foodpads with 0.2 ml of antigen-adjuvant emulsion for the induction of EAM. A peak increase in Bmax values of cardiac muscles of EAM was observed at day 18 (active phase), followed by a tendency to decrease until day 214 after immunization. However, there were no changes in Kd values at any stage of EAM. Thus, the present study indicates that β-AR densities change according to the stages of EAM.

Key words—rat experimental autoimmune myocarditis, cardiac muscle β-adrenoceptor, 3H-CGP12177, radioligand binding assay.

INTRODUCTION

Rat experimental autoimmune myocarditis (EAM) has been used as an animal model for human giant cell myocarditis1-4. EAM progresses in two stages: myocarditis (immune-mediated myocyte injury) in the active phase (during 10-45 days), and chronic heart failure in the late phase (45-210 days) after the immunization of myosin5. It is well known that the regulation of contractility in heart failure is controlled via the β-adrenoceptor (β-AR) by the β-adrenergic system6. Thus, the present study was planned to assess potentially important changes in β-AR densities (Bmax) and affinities (dissociation constant: Kd) in the myocardium by Scatchard analysis using the radioligand binding assay method at various stages of EAM induced by immunization with myosin.

MATERIALS AND METHODS

Immunization

Male Lewis rats were obtained from Charles River Japan Inc. (Atsugi, Japan). EAM was prepared using the method described previously7. In brief, purified cardiac myosin from the ventricular muscle of pig hearts was used as an antigen. The antigen prepared was dissolved at a concentration of 20 mg/ml in PBS containing 0.3 M KCl and mixed with an equal volume (1 ml) containing 11 mg/ml of Mycobacterium tuberculosis (Difco Laboratories, U.S.A.). Rats at 11 weeks of age were injected in both footpads with 0.2 ml antigen-adjuvant emulsion (n=110) and sacrificed on days 14, 18, 21, 24, 32, 49, 54, 68 and 214 after immunization. Fourteen-week-old male Lewis rats without immunization were sacrificed as controls.

Preparation of membrane-enriched fraction of hearts

The membrane-enriched fraction of EAM was prepared as described previously8. The thawed hearts were weighed, minced, and homogenized in 10 vol-
umes of 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, 5 mM MgCl₂, 10 mM EDTA, 3 mM phenylmethylsulfonyl fluoride (PMSF) and 2.5 mg pepstatin A with a polytron homogenizer. The homogenates were filtered through 4 layers of gauze and the filtrate centrifuged at 45,000 x g for 10 min at 4°C. The pellets obtained were resuspended in 50 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose and 5 mM MgCl₂ and centrifuged at 45,000 g for 30 min. The resultant membrane fractions were resuspended in the above buffer and frozen at -80°C until use.

Radioligand binding assay

Radioligand binding studies were performed according to the method described by Sato et al. Specimens were incubated in a buffer containing 50 mM Tris-HCl (pH 7.4), 0.25 M sucrose, 5 mM MgCl₂, 3 mM PMSF, and 2.5 mg L pepstatin A at 37°C for 60 min using 100 µg of membrane proteins. The total reaction volume was 500 µl. For saturation isotherms, membranes were incubated with varying concentrations (0.1 nM – 10 nM) of [³⁵S]CGP12177 in the absence (total binding) or presence (nonspecific binding) of 1 µM (+) propranolol. The reactions were stopped by filtration using Whatman GF/C glass fiber filters, and the radioactivity remaining in the filters was counted with a liquid scintillation counter. Kd and Bmax values of β-AR of EAM were determined using the Scatchard analysis. Protein contents were measured using the method described by Lowry et al. with bovine serum albumin as the standard.

Radioisotopes

(-)[³⁵S]CGP12177 (1.85 TBq/mmol) was purchased from New England Nuclear Corp. (Tokyo, Japan).

Statistics

The results of experiments are expressed as means± S.E. Significant differences were determined using Dunnett’s analysis; a P value of less than 0.05 was considered significant.

RESULTS

Bmax values of control male Lewis rats were 41.6±2.1 fmol/mg protein. Those values in myocardium with EAM were significantly increased to 123.3±16.7 fmoles/mg protein at 18 days after immunization with myosin. This was a 296% increase, which differed significantly from the control value. After this period, these numbers gradually decreased with time until 215 days after immunization, when Bmax values were lower than those of control values. (Fig. 1A) Kd (affinity) values in the myocardium of rats with EAM showed a small peak around day 25, but the change was not different from control values (Fig. 1B).

DISCUSSION

EAM has been used to clarify the pathophysiology of human myocarditis as an animal model for the failing human heart. In particular, it has been suggested that activation of the sympathetic nervous system during exercise or physiologic stress results in a dull response in augmenting the contractility. Bristow et al. suggested that alterations in myocardial adrenergic signal transduction play an important role in progressive heart failure in humans. Thus, it is of interest to assess changes in β-AR in the myocardium with EAM because determination of receptor numbers is one of the elements involved in the regulation of the β-AR-adenyl cyclase signaling pathway.

The present study showed a marked increase in Bmax values of β-AR during the active phase (days 18) of EAM, although in contrast the down-regulation of β₁-AR was observed in the acute phase of viral myocarditis. We have demonstrated that severe myocarditis occurs by extensive myocardial necrosis with inflammatory cell infiltrations, and interstitial edema developed consistently at this phase. Furthermore, a marked deterioration of hemodynamic parameters (reduction in mean arterial pressure, left ventricular systolic pressure, and peak positive dP/dt and and increase in LVEDP = left ventricular end-diastolic pressure) occurs during the active phase, and the hemodynamic changes recover to the control level by day 49 when acute inflammatory changes resolve. In addition, these results suggest that there is greater damage to heart functions and/or acute progressive heart failures against an immune reaction in the active phase than in the chronic phase. Thus, these increases in Bmax values of β-AR or increased stimulation of β-AR by adrenergic neurotransmitters like norepinephrine are needed for restoration or protection from persistent derangement of cardiac function during the acute phase.

In contrast to changes during the early phase, reductions in β-AR density were observed in the chronic phase. This reduction of β-AR density in the chronic phase resembles changes occurring during the failure of human hearts, suggesting that a decreased responsiveness to β-AR agonists accompanies chronic heart failure. These diminished β-AR densities may implicate the impaired cardiac
function. Further experiments will be needed however, for the determination and clarification of which subtypes of $\beta_1$- or $\beta_2$-ARs change in this model.

In conclusion, the different alterations in $\beta$-AR densities of cardiac muscles with EAM occur in the acute and chronic phases, suggesting that an increase of $\beta$-AR at the early phase is induced by inflammatory reaction to antigens, while heart failure at the late phase induces the deterioration of cardiac functions.

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REFERENCES