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ABSENCE OF AGE-RELATED CHANGES IN THE BINDING OF THE BETA ADRENERGIC ANTAGONIST ^{125}I-IODOHYDROXYBENZYLPINDOLOL IN RAT HEART

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Abstract. The effect of age on the density and the affinity of beta adrenergic receptors was determined in the hearts of Fischer 344 rats at three ages, 6, 12, and 24 months old. The binding of the beta adrenergic antagonist ^{125}I-iodohydroxybenzylpindolol (IHYP), was used to quantitate and characterize cardiac beta adrenergic receptors. The maximal number of binding sites (B_{max} = F moles/mg of protein) were 26.3±2.5, 25.4±0.99, and 24.5±2.4 and the dissociation constants (K_d = nM) were 0.166±0.014, 0.126±0.006, and 0.135±0.015 for 6, 12, and 24 months old animals, respectively. There were no significant differences among the three ages. These results support the contention that neither beta adrenergic receptor density or affinity changes with age in the ventricles of the rat heart. © 1987 Society for Experimental Biology and Medicine

Introduction. Age-associated changes in biochemical and physiological responses to various hormones and transmitters have been reported (1,2). In some cases, these changes appear to be associated with alterations in receptor concentrations.

In the cardiovascular system age-related changes have been reported in the response to adrenergic stimulation. Duckles (3) demonstrated a reduced response to transmural nerve stimulation of the rabbit ear artery in older animals while Goldberg et al (4) showed that the response to stimulation of the right cardiac sympathetic nerve is diminished in the heart of older rats. The inotropic and chronotropic response to isoproterenol diminishes with age (5-8).

Using [^{3}H]-dihydroalprenolol ([^{3}H]- DHA) as the radioligand, there was no effect of age on the affinity and density of cardiac beta-adrenergic receptors (9,10). However Fan and Bannerjee (11) using the same radioligand reported a decrease in the number of cardiac beta adrenergic receptors with age but there was no age-dependent change in affinity.

In the course of our studies designed to explore the age-related changes in adrenergic neural influences on the heart, a series of experiments were conducted to characterize the cardiac beta adrenoceptor in the heart of rats at various ages. Although Abrass et al (10); Guarnieri et al (9) and Fan and Banerjee (11) used [^{3}H]-DHA, we used [^{125}I]-iodohydroxybenzylpindolol (IHYP) because of its higher affinity and high specific activity (12,13). Since previous investigations on the effect of age on cardiac beta receptors reported different results using [^{3}H]-DHA we felt it would be important to report our results using a different ligand. Furthermore, since there appears to be disagreement regarding the selection of the radioligand to study beta receptor binding namely tritiated or iodinated compounds (14,15), comparisons of our results using iodionated pindolol to

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those obtained with tritiated dihydroalprenolol might bear on this controversy.

Materials and Methods. Fischer-344 male rats at three ages (6, 12, and 24 months old) were used in the experiments. In each age group, ten animals were employed. These rats were maintained under barrier conditions at Harlan Laboratories, Inc. (Indianapolis, Ind). In our institution, rats were housed in standard filtered cages, two per cage, in a temperature regulated environment (21±1°C), on a 12 hr light/dark cycle. The animals were fed ad libitum on a pasteurized rodent diet (19-26% protein, 4-6% fat) and on autoclaved water adjusted to pH 3. Maximum life span for Fischer-344 rats maintained under these conditions is approximately 35 months, with a mortality rate of about 50% at 29 months (16).

The rats were decapitated and the hearts were removed quickly and chilled in ice-cold buffer (Tris-isosaline pH 7.5). The ventricles were dissected free of the atria, fat and major vessels. The trimmed ventricles including the septum were minced in approximately 3 ml of 20 mM cold Tris-isosaline. The tissues were homogenized for 15 sec using a Tekmar Tissuemizer (setting 5-7) and then centrifuged at 20,000 x g for 10 minutes at 4°C. The pellets were resuspended in 5 volumes (based on wet weight of tissue) of Tris-isosaline. These crude membrane preparations were filtered through 3 layers of gauze and resuspended in 100 volumes of Tris-isosaline buffer. The protein content was approximately 30-60 ug protein per assay. Membrane preparations were stored at -70°C.

Assay of beta adrenergic receptor binding were carried out on washed crude membrane preparation of heart tissue employing a modification of the radioligand binding method reported by Minneman et al (13). IHYP (2200 Ci/mM) was used at 8 concentrations to determine the affinity and the total number of beta adrenergic receptors. The total assay volume was 250 ul in which IHYP at concentrations ranging from 250 pM to 20 pM, 500 uM GTP, 100 uM l-isoproterenol, 100 ul tissue preparation and 50 ul of BSA/ascrobic acid solution (0.001% Bovin Serum Albumin/ 1.4M ascorbic acid) were present. The assay volume was placed in disposable polypropylene tubes and set in an ice waterbath. Aliquots of the solution were incubated at 37°C for 25 minutes and rapidly filtered through glass fiber filters (Schleicher and Schuell No. 30) on a Brandel Cell Harvester followed by two additional washes using 8 ml of 10 mM cold Tris-isosaline. The filters were counted in a Beckman gamma counter at a counting efficiency of 65%. Specific binding was defined as the difference between binding of IHYP in the absence and presence of 100 uM l-isoproterenol. The density of binding sites (B_{max}) and their affinity (K_d) for IHYP were determined by linear regression analysis of saturation isotherm data, linearly transformed according to the method of Scatchard (17). To obtain a Scatchard analysis a computer program was used which was developed at the Department of Pharmacology, University of Pennsylvania.

Protein content was determined according to the Bradford method (18) using reagents prepared by Biorad. [^{125}I]-iodohydroxybenzylpindolol was purchased from New England Nuclear and GTP from Boehringer-Mannheim; all other chemicals were purchased from Sigma.

Differences in means were compared statistically using Analysis of Variance (ANOVA). A difference was considered to be statistically significant when the p value was less than 0.05.

Results. In Table 1 the kinetic parameters of specific binding of IHYP to beta adrenergic receptors are shown. B_{max} and K_d for IHYP were similar in the three age groups, ($p>0.05$). The density of binding sites were 26.3±2.5; 25.4± ±0.99 and 24.5±2.4 F mol/mg of protein and the K_d values were 0.166± 0.014; 0.126±0.006 and 0.135±0.015 nM, ($p>0.05$) respectively. Figure 1 represents a typical Scatchard analysis of IHYP binding to beta-adrenergic receptors in the ventricles; data from a single experiment derived from ventricles isolated from animals at each of the ages, i.e., 6, 12, and 24 month are depicted.

Discussion. In the present study, age-related changes in cardiac beta adrenergic receptor binding to IHYP in the

Table 1. Effect of age on the IHYP binding to the Beta-adrenergic receptors of ventricles in Fischer 344 rat heart

Ages (months)	Number of Animals	B_{max}* (Fmol/mg of protein)	K_d* (nM)
6	10	26.3±2.5	0.166±0.014
12	10	25.4±0.99	0.126±0.006
24	10	24.5±2.4	0.135±0.015

*The values indicate the mean ± SEM. No statistically significant differences were found in the total receptor density (B_{max}) or in the affinity (K_d) among the ages ($p>0.05$, ANOVA).

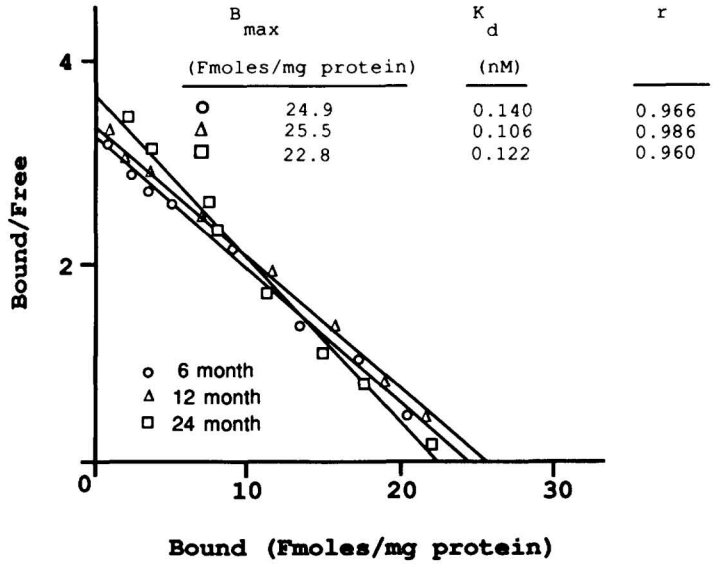

Figure 1. Scatchard analysis of IHYP binding to Beta-adrenergic receptors; each line represents the results of a single experiment using ventricles isolated from Fischer-344 rats of 6, 12, or 24 months old.

rat were not observed. The density and affinity of the receptor binding sites in the hearts of rats 6, 12 and 24 months of age were similar. These results are in agreement with those of Abrass et al (10) and of Guarnieri et al, (9) using [^{3}H]-DHA in Fischer 344 rats and Wistar rats respecitvely. They differ, however, from those reported by Fan and Banerjee (11), who also used [^{3}H]-DHA as the ligand. They reported that a greater number of binding sites are found in the myocardium of 3 months old Fischer 344 rats compared to those in hearts of 24 months old animals. Differences in the design of the experiments could account for the different results. Fan and Banerjee (11) anesthetized their animals with dimethyl ether before they were killed while in the present study and those of Abrass et al (10) and Guarnieri et al (9) decapitation was employed. Since ether is known to affect adrenergic activity (19) it is possible that the number of beta adrenergic receptors under the influence of ether would change with age. In any case it

appears for the most part, that changes in beta receptor number and affinity are not the major factors involved in the dimunition of beta receptors mediated responses of the heart with age.

It has been suggested that the defect in the beta adrenergic receptor system is located distal to protein kinase activation but proximal to the Ca^{2+}-troponin interacation (9). It may involve differences in the phosphorylation of proteins or altered calcium mobilization (6). An age-related defect in the coupling of the cardiac beta adrenergic receptor to the adenylate cyclase system has also been implicated. Kusiak and Pitha (20) concluded that there is diminished efficacy of the regulatory factors activating the catalytic subunit of adenylate cyclase. This is in agreement with O'Connor et al (21) and Narayanan and Derby (22).

On the basis of this investigation we could not distinguish between the suitability of iodionated or tritiated ligands for studying beta receptor binding characteristics since our results using an iodionated ligand closely parallels those using tritiated compounds.

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