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CARDIAC β-ADRENOCEPTORS DURING NORMAL GROWTH OF MALE AND FEMALE RATS

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1 A binding assay involving (−)-[^{3}H]dihydroalprenolol (DHA) and KCl-washed cardiac membranes was used to assess the numbers and affinities of β-adrenoceptors in hearts from male and female rats varying in age from about 2 weeks to 18 months.

2 Although female rats grow more slowly and attain lower adult weights than male rats, heart weights increased in approximate proportion to body weight with little sex difference.

3 As heart weight increased about three fold, β-receptors increased three fold. Since the number of myocardial cells is believed to be nearly constant during postnatal growth, the numbers of receptors/cell presumably increases similarly.

4 As heart weight increased, the number of β-receptors per g of tissue decreased according to the equation: total pmol/g = 4.33 − 1.43 × heart weight, equally in males and females.

5 Dissociation constants for DHA (2 to 4 nM) remained the same, and equal, in male and female rats during their growth.

6 An excellent correlation was found between the decline in β-receptors/g tissue during growth and the decline in the area of the external sarcolemma/g tissue. The data suggest that the number of receptors per unit area remains constant during growth, and thus that cell surface area is a major factor determining normal numbers of receptors per cardiocyte.

Introduction

Many factors are believed to influence the numbers of β-adrenoceptors of different tissues, including innervation, denervation, drugs which modify the degree of activation of receptors (Molinoff, Sporn, Wolfe & Harden, 1978), and abnormal levels of hormones, including corticosteroids (Wolfe, Harden & Molinoff, 1976), thyroxine (Williams, Lefkowitz, Watanabe, Hathaway & Besch, 1977) and oestrogens (Fregly, Thrasher, MacArthur & Kelleher, 1978). In the case of the heart, chronic stress and/or hypertrophic growth may also be important (Gelband & Bassett, 1973; Hein & Janke, 1977). β-Responsiveness is known to appear in the heart before its innervation (Pappano, 1977), and to remain in isolated cardiocytes (Hill-Smith & Purves, 1978). In the adult canine heart, β-receptors are distributed in close relation to the arrival of circulating adrenaline (Baker, Boyd & Potter, 1979). These observations suggest that both cellular factors governing the growth of myocardial cells, and levels of catecholamines, may modulate receptor numbers. In this study of male and female rats varying in age from about 2 weeks to 18 months, we have examined the relationship between normal growth and cardiac β-receptors in detail. The results suggest that cell surface area is a major factor determining normal numbers of β-receptors on cardiocytes.

Methods

Rats of the albino Sprague-Dawley strain were obtained randomly with respect to age over a 1 year period, from stock bred at this medical school and at the Charles River Breeding Laboratories, Inc. The oldest animals were ex-breeder males and females. Some of the considerable spread in results may derive from differences in animal care, and season. Results are given in terms of body or heart weight rather than age, because these indices gave the best comparisons between male and female hearts. It is known that female rats grow more slowly and reach lower mature weights than males. A circular provided by the Charles River Laboratories (No. 1R, dated July 1, 1977), gives approximate body weights at weekly intervals from 3 to 12 for males as 45, 85, 120, 145, 190, 230, 265, 295, 335 and 370 g, and for females as 40, 65, 100, 130, 155, 180, 200, 210, 220 and 230 g.

Animals were decapitated and their hearts were removed, rinsed in 0.9% w/v NaCl solution (saline), blotted and weighed. Each heart was homogenized in

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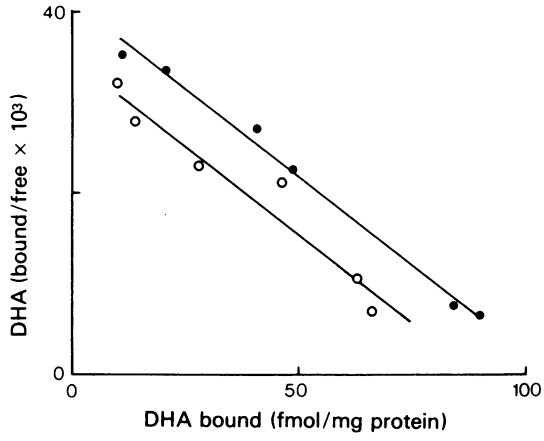

Figure 1 Scatchard plot of the binding of dihydroalprenolol (DHA) to KCl-washed membranes from 6 pooled male (●) and 6 pooled female (○) rat hearts. The animals weighed approximately 30 g each, and heart weights were 0.1 to 0.15 g.

10 ml of ice-cold 10 mM Tris–HCl buffer, pH 8, in a 45 ml Oak Ridge tube of known weight, with a Polytron P10ST blender at top speed for 30 s. Each homogenate was diluted with 30 ml of 1 M KCl, left on ice for 10 min, and centrifuged at 48,000 *gmax* for 10 min. Longer centrifugation did not yield more binding sites. Pellets were gently resuspended in 40 ml of buffer, resedimented, and finally dispersed again in sufficient buffer to yield membranes from 1 g of tissue in approximately 10 ml of suspension. Tubes were weighed, and the tube weight subtracted to determine the exact amount of suspension.

Binding sites for (−)-[^{3}H]dihydroalprenolol (DHA) were assessed as described by Baker, Boyd & Potter (1979). Most assays were carried out with 6 nM DHA. Examination of Scatchard plots prepared for animals of either sex and several ages (e.g., Figure 1) demonstrated that 6 nM DHA yields approximately 75% saturation of β-receptors. The values shown in the figures are not corrected to 100% saturation.

Results

Scatchard analyses of the binding of 0.3 to 10 nM DHA to cardiac membranes from 30 g and 700 g male rats and 30 and 300 g female rats yielded dissociation constants of 2 to 4 nM, in keeping with studies of canine heart tissue (Baker *et al.*, 1979). The most accurate data were obtained from the younger animals (Figure 1). There is thus no age or sex difference in the binding affinity for DHA to cardiac receptors from whole rat hearts.

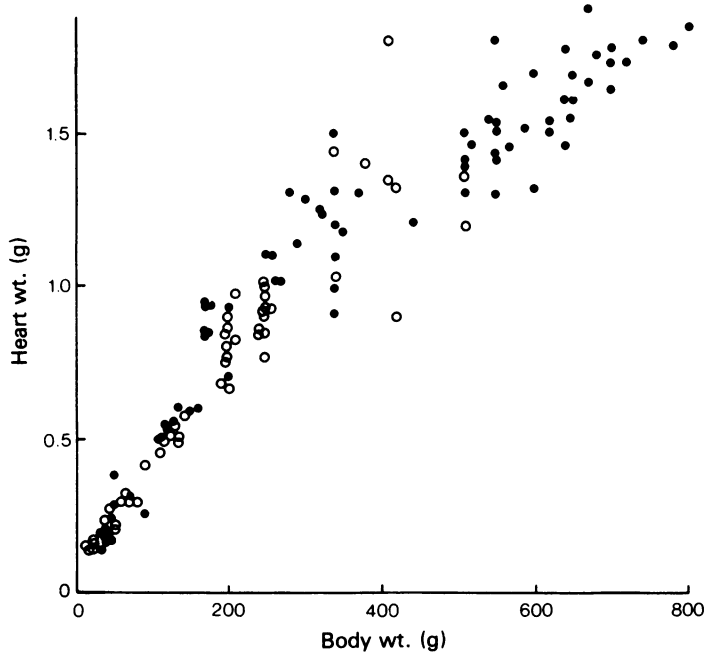

Figure 2 Relationship between body weight and heart weight in normal Sprague-Dawley rats. In this and the subsequent 3 figures, each point represents one male (●) or one female (○) animal.

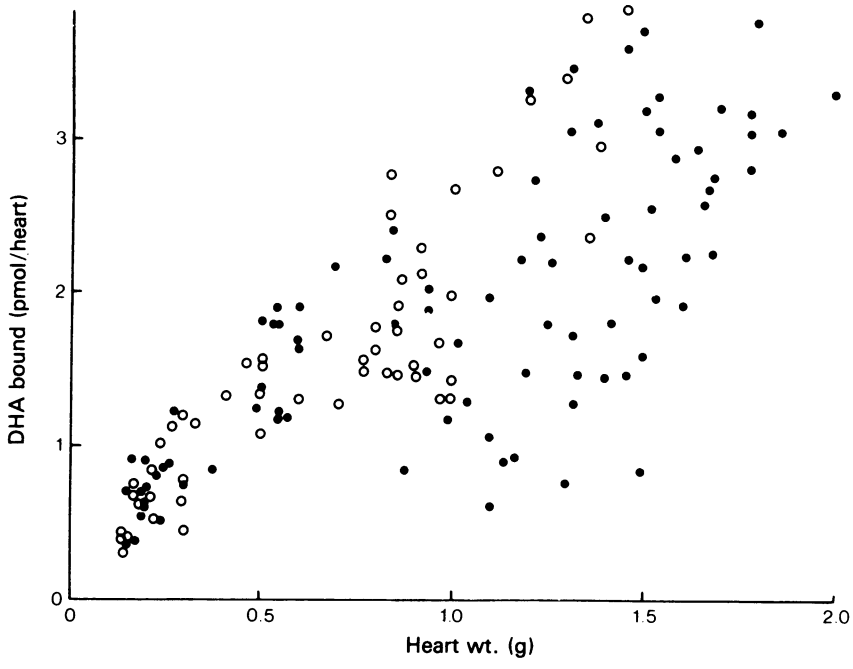

Figure 3 Increase in total cardiac β-adrenoceptors with increasing heart weight.

As the body weights of male and female rats increased, their heart weights increased proportionally and with no difference between the sexes, up to a heart weight of about 1 g (Figure 2). Thereafter, particularly in males which grow considerably larger, the heart weights increased more slowly than body weights.

Figure 3 shows that as heart weights increased, the total number of β-receptors per heart increased in approximate proportion, over at least a three-fold range. Again, up to a heart weight of 1 g there was little difference between the sexes; thereafter the number of receptors per male heart appeared slightly less than the number per female heart. Linear regression lines for all 91 male and 54 female hearts were not statistically different. A single line calculated for all animals had the characteristics: pmol/heart = 0.52 + 1.38 times heart weight in g, with a coefficient of determination of 0.56.

Although the total numbers of receptors increased with heart weight, the number per g of tissue fell during growth (Figure 4). Regression lines for pmol DHA binding sites per g of tissue for male and female hearts were virtually identical. One line calculated for all animals showed that pmol/g heart = 3.24 − 1.06 × heart weight, with a coefficient of determination of 0.42. With correction for 100% saturation of receptors the equation becomes 4.32 − 1.41 × heart weight. It may be noted that a 1 g heart has 2.9 pmol of β-receptors whether it comes from a male rat weighing about 250 g and 2 months of age, or a female weighing about 280 g and more than 4 months old.

Figure 5 shows the relationship between heart weight and the amount of DHA binding per unit protein in the KCl-washed particulate preparations which were assayed. The values for small, young hearts were clearly higher than those for hearts weighing 0.4 g or more, perhaps because small cardiac cells retain their structure and proteins better than larger cells, during homogenization. Beyond this weight, DHA binding per unit protein appeared relatively constant, with mean assay values (±s.d.) for males of 33 ± 15 (n = 79) fmol/mg protein, and 43 ± 13 for females (n = 39). This difference could occur by chance once in 5 such comparisons (Snedecor & Cochran, 1967), and is thus not statistically significant.

Discussion

Cardiac β-receptors increased about threefold during the period studied, and since the number of myocytes in rat hearts is known to change little during this period (Page & McCallister, 1973; Korecky & Rakusan, 1978), the number of receptors per cell must also have increased about threefold. The number of

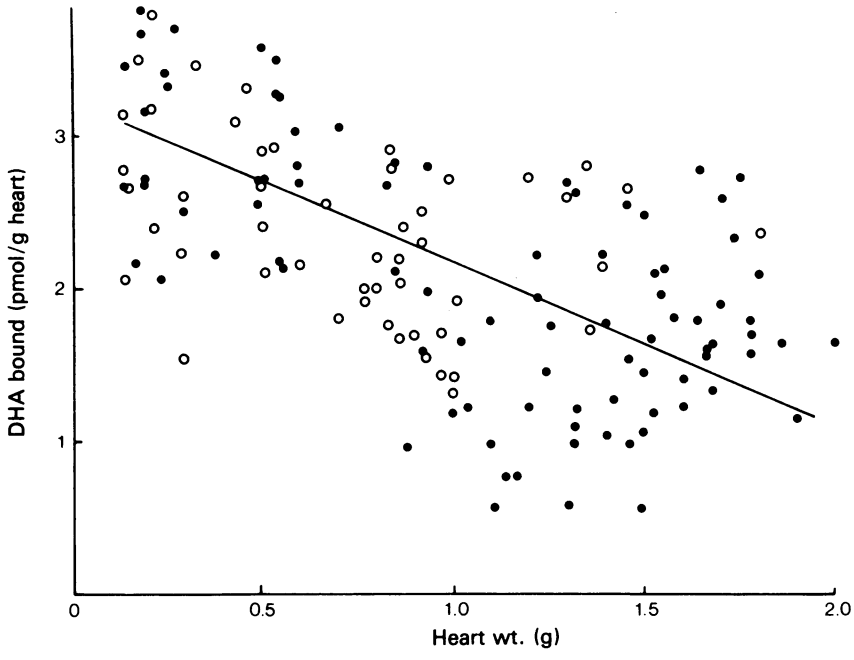

Figure 4 Decrease in β-receptors per g of tissue with increasing heart weight. The line was calculated by the least squares regression method.

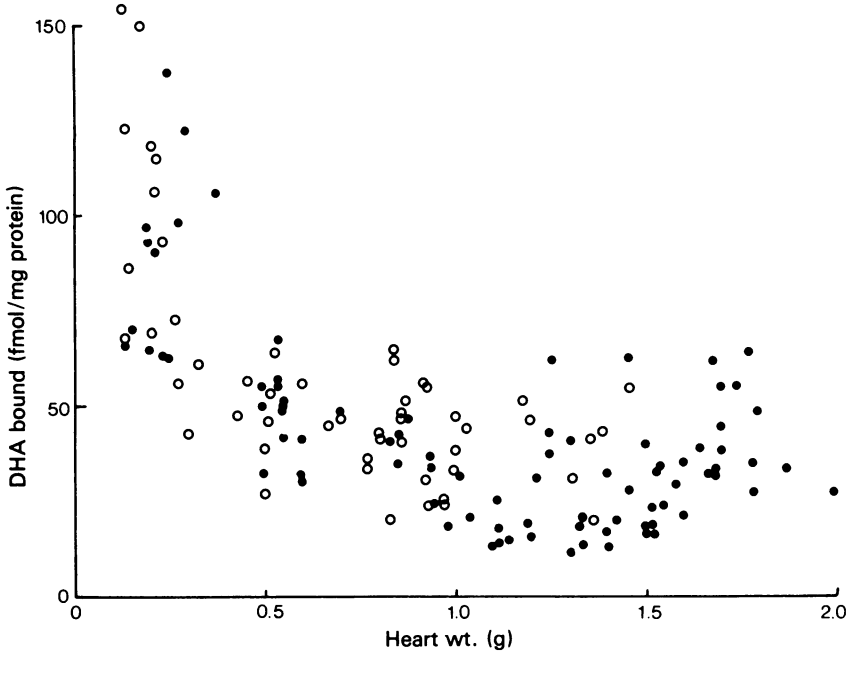

Figure 5 Relationship between β-receptors per unit membrane protein and heart weight.

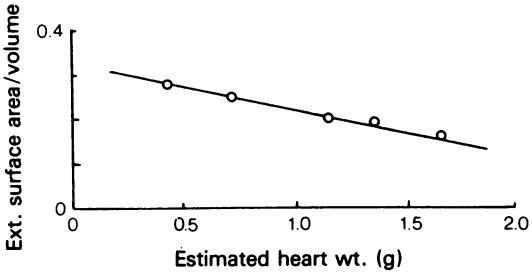

Figure 6 Relationship between decreasing β-receptors/g of tissue, during growth, and the simultaneous decline in myocyte external surface/volume ratio. The line has the same slope as that of Figure 4. The points are calculated from data given by Korecky & Rakusan (1978) as described in the Discussion.

sites per unit heart weight or volume, however, clearly decreased with growth. Since the total surface (external sarcolemma + T-tubular membrane) to volume ratio in growing myocardial cells remains constant, and there is little change in the proportion of cardiac volume occupied by cells (Page & McCallister, 1973), it appears that β-receptors do not increase in proportion to total cell surface. Nonetheless, β-receptors are in cardiac membranes, and the ratio of receptors to membrane protein was nearly constant for male and female hearts over 0.4 g. We have therefore attempted to correlated β-receptor numbers with the area of the external sarcolemma only, which constitutes a decreasing proportion of the total membrane in growing myocardial cells (Page & McCallister, 1973). Data for Figure 6 were taken from Table 1 of Korecky & Rakusan (1978), which gives average cell lengths and widths for freshly isolated left ventricular cells from male Sprague-Dawley rats, at five stages of body weight. Corresponding heart weights were estimated from a smooth curve drawn through the points for male animals of Figure 1 of this paper, and the ratio of cell surface to volume was calculated, assuming (as did Korecky & Rakusan) cylindrical cell shape. Our derived surface/volume ratios are slightly lower than the ratio of 0.3 determined from stereographic analysis of electron micrographs of left ventricular cells from 200 to 220 g female rats (Page & McCallister, 1973). Figure 6 shows an almost perfect correlation between the decline of β-receptors/g tissue and the decline in cell surface/volume ratio with cardiac growth. While the data can undoubtedly be refined by further work, the available results suggest that the number of β-receptors per unit area of external sarcolemma remains constant during normal cardiac maturation. We are surprized that this correlation does not include the area of cardiac T-tubules, which constitutes about one quarter of the total surface (Page & McCallister, 1973), since we have previously shown that T-tubules isolated from skeletal muscle are rich in β-receptors and isoprenaline-activated adenylate cyclase (Caswell, Baker, Boyd, Garcia & Potter, 1978), and would have predicted that cardiac T-tubules were similarly endowed.

Since there was no significant difference in the numbers or binding affinities of cardiac β-receptors in male and female rats, we conclude that male and female sex hormones present during normal growth do not exert different regulatory influences, if any, on these receptors. However, it has been reported that supranormal amounts of female sex hormones attenuate cardiac responses to isoprenaline in female rats (Fregley *et al.*, 1978). The question thus remains as to whether alteration of the norm modifies receptors or some subsequent step in β-responsiveness.

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