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EFFECT OF A HIGH CARBOHYDRATE DIET ON CARDIAC ALPHA-1 AND BETA ADRENOCEPTORS

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Short-term (2 weeks) administration of a high-fructose diet to euthyroid Sprague-Dawley rats results in a significant (18%) increase of cardiac β-adrenoceptors without any change in their affinities or their distribution between the plasma membrane and a vesicular fraction. A much smaller increase occurs in the number of α_1-adrenoceptors (8% higher than in rats fed a regular diet). The high-carbohydrate diet induced a 39% increase in β-adrenoceptor numbers/heart in hypothyroid animals and a 19% increase in the total number of α_1-adrenoceptors. These results strongly suggest that changes in cardiac performance after dietary manipulations may be mediated, in part, through enhancement of adrenergic pathways.

It has been known for some time that diets high in carbohydrate improve cardiac function in humans but the mechanisms involved have remained elusive. Recently, evidence has accumulated that several processes important in the regulation of contractile function can be influenced by dietary manipulations.[1-4] Specifically, myosin Ca^{2+} ATPase activity and myosin isoenzyme distribution is enhanced in both euthyroid and hypothyroid rats fed a carbohydrate-rich diet and this enhancement is synergistic with the effects of thyroid hormones.[1,3] Furthermore, methylpalmoxirate (which inhibits fatty acid oxidation and stimulates glycolytic flux) reverses the decreases in myosin ATPase activity and isoenzyme redistribution seen in the diabetic rat heart.[2] It has been suggested, therefore, that stimulation of glycolytic flux by the high carbohydrate diet generates signals which may be amplified by thyroid hormones. A similar synergism has been observed in the induction of hepatic malic enzyme and in $mRNA_{S14}$ and has been attributed to changes at both the transcriptional and post-transcriptional level.[4-6] Changes in the composition and properties of nuclear

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chromatin as a result of high-carbohydrate feeding have also been described.[7]

These observations strongly suggest that metabolic changes induced by a high-carbohydrate diet generate signals which may profoundly affect the performance of the heart. This effect may not be limited to the contractile proteins: in the present communication, we provide evidence for a significant effect on cardiac α_1- and β-adrenoceptors.

MATERIALS AND METHODS

Experiments were carried out on euthyroid Sprague-Dawley rats (Bio-Lab, Inc., St. Paul, MN) weighing 300-350 g. Control animals were placed on either regular or high-fructose diet (ICN) for 2 weeks. The regular diet contained 47% complex carbohydrates and the ICN diet, 60% fructose. In a separate group of animals, hypothyroidism was induced by adding 0.025% methimazole to the drinking water for 4 weeks. Animals were then randomized to either the regular or the high-carbohydrate diet group. At sacrifice, heart weight, body weight and heart/body weight ratios were obtained.

Cardiac β_1- and α_1-adrenoceptors were assayed in the plasma membrane and vesicular fractions prepared by a slight modification of previously described techniques.[8] Briefly, hearts were minced in 5 volumes of cold 50 mM Tris-Hcl-5mM EDTA (pH 7.5) and homogenized with a Polytron PT-20 homogenizer at half-maximal speed for 20 sec. The homogenates were centrifuged at 500 xg for 10 min. and the supernatants were passed through gauze prior to centrifuging at 40,000 xg for 15 min. The resultant pellet was washed twice and was used as the membrane fraction. The supernatant was centrifuged at 150,000 xg for 1 hr. and the resultant pellet was used in the vesicular fraction. For determination of β-adrenoceptors in the membrane and vesicular fractions, the assay medium contained 50 mM Tris-HCl (pH 7.5) - 5 mM $MgCl_2$, 6 nM [^{3}H]DHA and 0.1 - 0.2 mg protein (determined by the method of Lowry, et al.)[9] in a total volume of 0.5 ml. Incubations were carried out at 37°C for 15 minutes and terminated by filtering through Whatman GF/C filters, washing X3 with 5 ml cold Tris-Mg buffer and drying the filters before counting. Nonspecific binding was determined in the presence of 1 μM propranolol. α_1-Adrenergic receptors were determined using subcellular fractions. Incubations were carried out at 37°C for 15 min. in a medium containing 50 mM Tris-HCl (pH 7.5) - 5 mM $MgCl_2$, 0.025 - 20 nM [^{3}H]prazosin (sp. act 24.4 Ci/mmol, Amersham/Searle), and 0.1 - 0.3 mg membrane protein in a total volume of 0.5 ml. At the end of the incubation time, 2 ml of cold buffer were added to each tube and the contents passed through Whatman GF/C filters. The filters were washed three times with 5 ml of cold buffer, dried, and transferred to scintillation vials for counting. Nonspecific binding was determined in the presence of 10^{-5} M phentolamine and averaged 5% of the total. Maximal numbers of receptors and affinities were calculated from Scatchard plots[10] of the binding data.

RESULTS AND DISCUSSION

High carbohydrate diet in euthyroid rats did not significantly affect heart weights (0.93 ± 0.02 gm vs. 0.92 ± 0.03 in regular-diet controls) or heart/body weight ratios (2.55 ± 0.06 mg/g vs. 2.50 ± 0.05 mg/g in controls). Nevertheless, it induced substantial changes in the densities of membrane-bound cardiac adrenoceptors. Since previous studies[11,12] have indicated that a substantial proportion (30-50%) of β-adrenoceptors may be located intracellu-

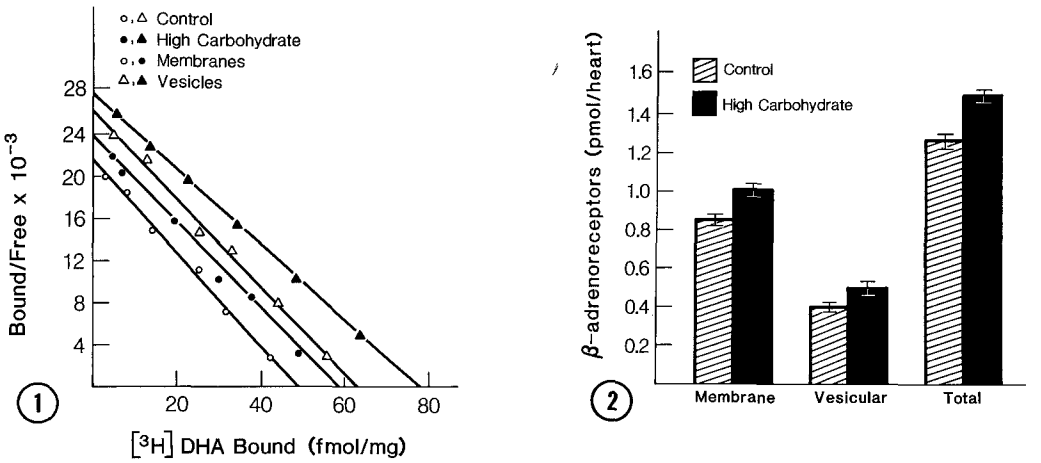


Fig. 1. Scatchard plot of [^{3}H]dihydroalprenolol binding to cardiac membranes and vesicular fractions from animals on either regular or high-carbohydrate diets.

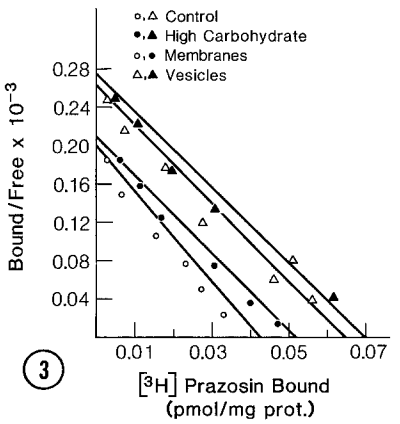
Fig. 2. Recovery of β-adrenoceptors from membranes and vesicular fractions of rats on either regular or high-carbohydrate diets. Results represent mean ± SE for six comparisons.

larly and that the relative distribution of adrenoceptors between the plasma membrane and vesicular fraction may change under physiological and pathological conditions, we examined the effect of diet on β-adrenoceptors in both cellular pools. The vesicular fraction is devoid of plasma membrane markers and contains β-adrenoceptors uncoupled from adenylate cyclase,[12] presumably as a result of sequestration (e.g., during the process of agonist-induced desensitization). Figure 1 shows a Scatchard plot of [^{3}H]dihydroalprenolol binding data for cardiac membranes and vesicular fractions from euthyroid animals on either regular or high carbohydrate diet. The density of β-adrenoceptors is higher in the vesicular compared to the membrane fraction for both diet groups, a finding compatible with the suggestion that the vesicular fraction represents a specialization of the membrane containing sequestered receptors. No significant differences exist in the affinities of the β-receptors between fractions or diet groups. However, receptors density is substantially higher in the high carbohydrate group for both membrane and vesicular fractions.

We then examined whether the distribution of β-adrenoceptors between the vesicular and membrane fractions was influenced by the diet. Figure 2 compares

the recovery of β-adrenoceptors (receptor density x amount of protein in the relevant fraction recovered per heart) in the two experimental groups. A comparable portion of the total β-receptor complement is recovered in the vesicular fraction (32 ± 0.2% in the control and 33.4 ± 0.3% in the high-carbohydrate group). However, the total number of cardiac β-adrenoceptors is about 18% higher in the animals fed the high-carbohydrate diet.

In contrast to β-adrenoceptors, cardiac α_1-adrenoceptors were less influenced by dietary manipulations. Figure 3 shows a Scatchard plot of the [^{3}H]prazosin binding data for a representative experiment. The density of α_1-adrenoceptors is higher in the vesicular than in the membrane fraction for both animal groups, similar to the observation for the β-adrenoceptors. There is a small, about 8%, increase in α_1-receptor density in both subcellular fractions of the high-carbohydrate groups without a change in receptor affinity. The proportion of α_1-adrenoceptors recovered in the vesicular fraction (Figure 4) is somewhat higher than that for β-adrenoceptors but does not differ in the two experimental groups (40.3 ± 1.1% in the high-carbohydrate group and 39 ± 0.3% in the control group). There is, however, a small, statistically signifi-



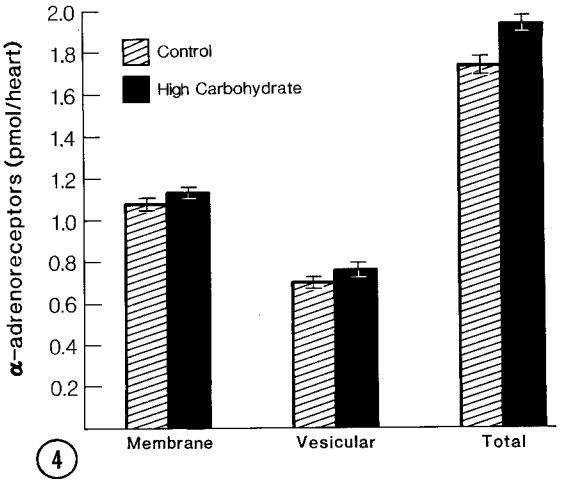


Fig. 3. Scatchard plot of [^{3}H]prazosin-binding to cardiac membranes and vesicular fractions from rats on either regular or high-carbohydrate diets.

Fig. 4. Recovery of α_1-adrenoceptors from the membrane and vesicular fractions of rats on either regular or high-carbohydrate diets. Results represent mean ± SE for six comparisons.

cant increase in the total number of α_1-adrenoceptors recovered from hearts of carbohydrate-fed animals.

It is not clear why dietary manipulation has a quantitatively different effect on β- and α_1-adrenoceptors in the rat heart. Our explanation might be the relatively short (2 weeks) duration of the exposure to the diet which might influence the degree of accumulation of proteins with a different half-life. In the young rat, turnover rate for cardiac β-adrenoceptors has a half-life of approximately 4 days[13] while comparable data for α_1-adrenoceptors are not available. The results are, however, relevant to the proposed synergism between carbohydrate feeding and thyroxine administration, which has suggested to some a convergent pathway in the action of these two interventions.[1,2] It is well-known that thyroid hormones have disparate effects on cardiac β- and α_1-adrenoceptors, resulting in higher density of the former and lower of the latter.[14] Our results, therefore, reveal a discrepancy in the directional changes of cardiac adrenoceptors induced by diet and thyroid hormones. In order to further investigate this aspect, we compared the effects of a high carbohydrate diet on hypothyroid animals. Induction of a hypothyroid state has a profound effect on the cardiac β-adrenoceptors with reduced membrane-density (36 ± 0.03 fmol/mg prot vs. 47 ± 0.04 fmol/mg in controls, $p < 0.01$), as well as total number recovered per heart (0.72 ± 0.06 pmol vs. 1.15 ± 0.08 pmol in euthyroid controls, $p < 0.01$) without a change in the proportion of β-adrenoceptors in the vesicular fraction. After two weeks on the high-carbohydrate diet, there is a 39% increase in the total number of β-adrenoceptors (0.99 ± 0.07 pmol/heart) compared to animals on the regular diet but, in addition, there is a 19% increase in α_1-adrenoceptors (1.21 ± 0.08 pmol/heart compared to 1.02 ± 0.07 pmol/heart in rats on a regular diet). The density of β-adrenoceptors in the membrane fraction is increased by 34% compared to the control group, while that in the vesicular fraction is increased by 36%. In contrast, α_1-adrenoceptor density in the plasma membrane is increased by 10% and that in the vesicular fraction by 20%. The higher number of the α_1-adrenoceptors recovered in the hypothyroid-high carbohydrate group, however,

reflects to a large extent the difference in heart weights between the two groups (0.721 ± 0.17 gm vs. 0.655 ± 0.05 gm in the control group, $p < 0.05$), while heart/body weight ratios were unchanged (2.03 ± 0.02 mg/g vs. 1.95 ± 0.02 in controls). The number of α_1-adrenoceptors per gm tissue, therefore, was not significantly different in the two diet groups, while β-adrenoceptors per gm tissue were still 19% higher in the high-carbohydrate group. This is similar to the increase seen in euthyroid animals.

The results of this study expand the list of cardiac proteins modulated by a high carbohydrate diet to include β- and α_1-adrenoceptors. In view of the significant role adrenergic pathways play in regulating calcium fluxes and the interaction of contractile proteins in the heart, there are obvious functional implications of our findings. Experiments are now underway to determine whether dietary manipulations can influence the decline of cardiac β-adrenoceptors under pathologic conditions, e.g., hypertrophy.[15]

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