BRIEF COMMUNICATION

Cholesterol Synthesis and Metabolism as a Function of Unpredictable Shock Stimulation

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PARÉ, W. P., B. ROTHFELD, K. E. ISOM AND A. VARADY. Cholesterol synthesis and metabolism as a function of unpredictable shock stimulation. PHYSIOL. BEHAV. 11(1) 107–110, 1973.—Rats fed a high lipid diet and also exposed to unavoidable and unpredictable grid shock for 2 and 8 days showed higher levels of accumulated chemical cholesterol in aorta, kidney, liver and serum as compared to control rats fed only the high lipid diet. Thirty days of shock stress produced similar results except that kidney tissue revealed lower and nonsignificant levels of accumulated cholesterol. A differential cholesterol clearing mechanism for the various tissues is postulated to explain the relative vulnerability of aorta, liver and kidney to stress-induced cholesterol deposition.

INCREASES in serum cholesterol have been correlated with stressful life situations from workers subjected to cyclic occupational stress [3] as well as from hospitalized patients [5,13]. Medical students also manifest increases in serum cholesterol levels during examination periods [2, 4, 9, 11]. These observations are significant since coronary atherosclerosis appears to be related to a disorder in lipid metabolism and especially serum cholesterol concentration. The significance of these postulated relationships is accentuated if we include the relationship of atherosclerosis to myocardial infarction. While the mechanism for atherosclerosis is not clearly understood, we can assume, on the basis of the reports mentioned above, that environmental stress does have some impact on this disorder. We can hypothesize that environmental stress which precipitates hypercholesterolemia will have a direct bearing on the deposition of fat in various body tissues and on the incidence of coronary heart disease.

The human studies previously cited do not answer two important questions. First, do stressful life events effect the deposition of fat in tissue? Second, does prolonged or chronic stress have a differential effect on cholesterol metabolism and synthesis? Human studies preclude the investigation of fat deposition in various organ systems and do not lend themselves to chronic stress investigations. Therefore, an animal analogue is proposed for the present investigation. Rothfeld, Karmen and Hunter [8] reported an appropriate experimental procedure in which rats fed a high lipid diet for 36 days demonstrated elevated levels of cholesterol in aorta, kidney and liver. On the other hand, Weiss [10], and Caul, Buchanan and Hays [1] demonstrated that environmental stress in the form of unpredictable grid shock had very aversive effects on the restrained rat when stress effects were measured in terms of body weight loss and stomach ulcer pathology. Price [7] reported similar effects of unpredictable shock with unrestrained rats. In the present study, the high lipid diet technique of Rothfeld, et al. [8] was utilized in conjunction with the environmental stress schedule of Weiss [10] and Caul, et al. [1] in order to answer the questions posed above. More specifically the hypothesis investigated was that rats maintained on a high lipid diet and subjected to unpredictable shock stress would manifest higher cholesterol levels in serum, aorta, kidney and liver, as compared to nonstress controls and that these differences would be accentuated as a function of the length of stress exposure.

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METHOD

Animals and Apparatus

The animals were 72 male Sprague-Dawley rats weighing 180–200 g at the beginning of the study. The apparatus consisted of 12 Lucite grid boxes 73 cm long, 27 cm wide, and 30 cm high. The tops were made of plywood and served as the entry lid to the apparatus. The grid floors were composed of stainless steel rods spaced 1.25 cm apart. A graduated drinking tube was mounted outside at one end of each box with the drinking spout protruding into the cage. A spillproof food hopper was situated at the opposite end of the cage. The grid floors were electrified with an a.c. constant current Applegate stimulator. Presentation of sound and shock stimuli was accomplished by two film-tape programmers and associated relay circuitry.

Procedure

Twenty-four animals were handled at one time. Rats were individually placed in the grid boxes for a 7-day habituation period to familiarize the animals with the food hoppers. Water was always available. The only food which was available during the entire experiment was the high lipid diet. The composition of the diet has been described previously [7]. After habituation, rats were matched for body weight and randomly assigned to either the experimental or control group. On Day 8 the stress schedule was started for animals in the experimental groups. The stress schedule consisted of tones and grid shocks presented on a VI 3-min schedule. The tone stimulus was a 10-sec, 1200 cps tone which was 10 db above the 74 db (re 0.0002 dyne/cm^2) ambient noise level of the room white noise masking system. The shock stimulus was a 0.5 sec, 1 ma shock presented via the grid floor. Tone stimuli and shock stimuli were presented completely independent of one another. No contingency existed between tone stimuli and shock stimuli. The stress schedule was in effect daily from 8 a.m.—2 p.m., and 8 p.m.—2 a.m. Control rats remained in the grid boxes but were never subjected to the stress schedule. Food and water consumption and body weight were recorded daily. To control for food intake between experimental and control animals, the control rats were individually matched with an experimental mate and the amount of food consumed by the experimental rat in one day was subsequently fed to its control mate on the next day. In this manner, control rats lagged behind the experimental rat by 24 hr as far as the experimental protocol was concerned. To measure cholesterol deposition and synthesis in the rat as a function of varying durations of environmental stress, the procedure outlined above was carried out three times with different stress-duration periods of 2, 8 and 30 days.

In order to obtain estimates of cholesterol deposition and synthesis, various assay procedures were used. The high lipid diet was taken away from all rats 72 hr before the termination of the various stress duration periods. Food removal occurred at 8 a.m. Twenty-four hr later, half of the experimental and control rats were given 40 µc of Na acetate-2^14C in saline intravenously. The other half in each group were fed one Purina Chow pellet impregnated with 25 µc of 4-^14C cholesterol. The intravenous treatment was designed to provide an estimate of synthesis and degradation of newly-formed cholesterol in various organs at the time of Na acetate-2^14C administration. The oral administration of 4-^14C cholesterol was designed to provide an estimate of the sum of absorption, distribution and degradation of cholesterol at the time of the oral administration. The oral and intravenous treatments started at 8 a.m. and took less than 2 hr to complete. Immediately after completion of the oral and intravenous treatments the high lipid diet was returned to all animals. Forty-eight hr later all rats were killed. Aortas, kidneys and livers were cleaned of connective tissue, and these organs as well as the serum were analyzed for chemical cholesterol as described by Hawk, Oser and Summerson [6]. The chemical cholesterol provided an estimate of accumulated cholesterol deposition in organs and serum analyzed. These tissues were also minced and extracted with isopropanol. Cholesterol esters were hydrolyzed with KOH and the cholesterol was precipitated with digitonin. Aliquots of the precipitate were analyzed for digitonin-precipitated radioactivity by liquid scintillation counting. Thus, the chemical cholesterol provided estimates of accumulated cholesterol deposition in tissue and serum, whereas the oral treatment provided an estimate of the algebraic sum of absorption, distribution and degradation of cholesterol. The intravenous treatment gave an estimate of synthesis and degradation of newly-formed cholesterol in the tissues studied. Each of the radioactive treatments provided data at one point in time, i.e., at the time the animals were killed.

RESULTS AND DISCUSSION

A two-factor (2X3) analysis of variance design ([12], p. 241) was used to analyze the two factors of experimental treatment and stress duration for all dependent variables. With this design the significance of simple effects was evaluated with an F-test and multiple group comparisons within factors were evaluated, when appropriate, by a Tukey (a) test ([12], p. 87).

Analysis of body weight differences failed to reveal any significant differences between experimental and control rats exposed to either 2, 8 or 30 days of stress.

The results of the chemical cholesterol assays, which are an indication of the endogenous accumulated levels of cholesterol in aorta, kidney, liver and serum, are shown in Fig. 1. In practically all the comparisons between experimental and control groups, the experimental rats displayed higher cholesterol levels in serum and aorta, kidney and liver tissue. The analysis of variance for cholesterol levels in aorta tissue resulted in a significant difference for the main effect of treatment conditions (F = 27.14, df = 1, 66, p<0.01). As Fig. 1 illustrates, aortas of experimental rats displayed higher levels of chemical cholesterol for all three stress—duration periods as compared to controls.

Analysis of cholesterol levels in kidney tissue revealed a significant difference for the main effect of stress duration (F = 242.79, df = 2, 66, p<0.01). This difference was attributable to the higher kidney cholesterol values of both experimental and control rats after 8 days of stress (Tukey (a) test, p<0.05), as compared to 2 and 30 days of stress. The significant main effect of treatment conditions in this same analysis (F = 6.69, df = 1, 66, p<0.025) was attributable to the higher kidney cholesterol values of experimental rats as compared to controls after 8 days of stress (F = 15.21, df = 1, 66, p<0.01). Kidney cholesterol differences between experimental and control groups were not significant after 2 or 30 days of stress.

Liver cholesterol deposition increased for both treat-
ment groups as a function of longer exposure to stress and these changes were reflected by the significant F-value obtained for the main effect of stress duration in the analysis of variance of the liver cholesterol data ($F = 59.95$, $df = 2, 66, p<0.01$). Experimental rats had higher liver cholesterol values as compared to controls (main effect of treatment conditions: $F = 9.55$, $df = 1, 66, p<0.01$).

Evaluation of simple effects indicated that this significant difference occurred only after 8 and 30 days (8 days stress: $F = 4.14$, $df = 1, 66, p<0.05$; 30 days stress: $F = 17.0$, $df = 1, 66, p<0.01$), and not after 2 days of stress.

Analysis of serum cholesterol data revealed a significant F-value for the main effect of treatment conditions ($F = 12.38$, $df = 1, 66, p<0.01$). Evaluation of simple effects
showed that experimental rats had higher serum cholesterol values as compared to controls after 8 and 30 days of stress (8 days of stress: F = 4.54, df = 1, 66, p<0.05; 30 days stress: F = 26.84, df = 1, 66, p<0.01), but not after only 2 days of stress. However, a significant interaction term (F = 10.25, df = 2, 66, p<0.01) for this same analysis of variance indicated that the effect in serum cholesterol values of control rats at 8 days of stress from the higher values for controls after 2 days of stress. Multiple group comparisons of the three control groups for the three stress–duration periods showed that serum cholesterol values for control rats after 8 days of stress were significantly lower than values recorded for control rats after 2 days of stress (Tukey (a) test, p<0.05).

Figure 2 illustrates the incorporation of intravenous Na acetate-2\(^{14}\)C into cholesterol and the deposition of fed \(^{14}\)C-cholesterol into cholesterol. Although experimental rats, as compared to control rats, showed higher cholesterol levels in all three tissues analyzed, the incorporation of intravenous Na acetate-2\(^{14}\)C was not significantly different between experimental and control groups in the liver and kidney for any of the three time periods observed. However, significantly higher levels in aortas were obtained for stressed rats after 2 days (F = 8.79, df = 1, 66, p<0.01) but not after 8 or 30 days of stress.

The kidney and liver of experimental rats had higher values of fed 4\(^{14}\)C cholesterol but these differences were not statistically significant. Fat deposition was greater in the aorta of stressed rats but this difference was significant only after 30 days of stress (F = 18.89, df = 1, 66, p<0.01). The fact that few significant differences were observed between treatment groups for the radioactive assays and that numerous differences were recorded for chemical cholesterol is interesting. Since the radioactive assays measured primarily cholesterol absorption, these results indicated that the effect of the experimental treatment was not on the rate at which the various tissues absorbed cholesterol, but on the ability of the tissues to clear cholesterol. It would seem that shock stress does not effect a tissue’s ability to absorb cholesterol, but it does effect its ability to release and clear cholesterol.

Gastrointestinal pathology has been demonstrated in rats subjected to unpredictable shock stress. In the present case, a similar schedule resulted in a significant hypercholesterolemia in stressed rats. We can suggest, on the basis of the observed data, that the aorta, kidney and liver possess differential cholesterol clearing mechanisms. The aorta may be most vulnerable. Cholesterol accumulated in the aorta after only 2 days of stress and remained significantly higher for 8 and 30 days, whereas at least 8 days of stress were required for significant elevations of cholesterol in the other organ systems. On the other hand, the hypothetical clearing mechanism of the kidney seems superior to that of the liver and aorta. Although significantly higher cholesterol levels were observed in the kidney after 8 days of stress, results after 30 days of stress did not reveal these increases. This suggests a greater adaptive capability of the kidney as compared to the aorta and liver to clear cholesterol.

These results supported the previous reports on life stress and hypercholesterolemia. The aorta data also appeared to strengthen the purported link between environmental stress and atherosclerosis. The occurrence of environmental stressful situations can apparently lead to significant changes in tissue cholesterol concentration. It is tempting to suggest that the experimental procedure outlined in this report represents an animal model, or experimental technique, for the study of human hypercholesterolemia, but such a suggestion would be unwarranted. While the relationship between hypercholesterolemia and tissue lipid deposition still poses many difficult questions, the present study would suggest that environmental stress may have some input in the development of vascular disorders.

REFERENCES


