DISTRIBUTION OF ACTIVITY OF ENZYMES SYNTHESIZING AND HYDROLYZING CHOLESTEROL ESTERS IN THE HUMAN AORTA

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Activity of acyl-CoA-cholesterol-acyltransferase was determined in various parts of the aorta of persons of different ages, in some of whom cholesterol esterase activity was found. No relationship was discovered between the activity of these enzymes and the type of atherosclerotic lesion.

KEY WORDS: atherogenesis; cholesterol esters.

The vascular wall is a tissue in which the main events of atherogenic degradation directly connected with lipid metabolism and, in particular, with the regulatory mechanisms of biosynthesis and hydrolysis of cholesterol esters, take place.

In this context it is interesting to study how activity of enzymes of opposite function (those synthesizing and hydrolyzing cholesterol esters) changes in an area of the aorta, especially in parts affected and not affected by atherosclerosis, in different age groups, and also in different layers of the intima, media, and adventitia. The presence of acyl-CoA-cholesterol-acyltransferase (ACAT) and of cholesterol esterase in the vascular wall was demonstrated previously [3, 7]. ACAT activity is also known to be higher in atherosclerotic areas of the aorta than in unaffected areas [4].

EXPERIMENTAL METHOD

Human aortas (3-12 h after death) and blood vessels of Wistar rats obtained immediately after slaughter of the animals or, in the control series, after the cadavers had been kept for 3-18 h at 20°C, were used. The tissues were ground in glass homogenizers with 0.2 M Tris-HCl, pH 7.1, and 1 M EDTA as extracting agents, after which they were centrifuged (4°C, 10,000g, 10 min). The supernatant was used as the source of enzymes. Protein was determined by Lowry's method [5]. Supernatant heated to 100°C (5 min) served as the control. Palmityl-[^{14}C]-CoA(22 μCi/mmole), cholesterol-[1-^{14}C]-palmitate (17.4 mCi/mmole), and [^{14}C]-cholesterol (55.6 mCi/mmole) were used as substrates. To 0.2 ml of the supernatant was added 0.2 ml of a solution containing 6 μM ATP, 0.4 μM coenzyme A, 4 μM $MgCl_2$, 2 μM NaF and 0.4 μM dithiothreitol, after which labeled cholesterol (900 pM) or cholesterol-palmitate (237 pM) in acetone or palmityl-CoA (178,500 pM) in water (50 μl of each) were added [6]. The reaction was stopped after incubation (3 h, 37°C) by the addition of 1 ml ethyl acetate or chloroform, the sample shaken for 3 min, and the organic phase separated and applied to a "Silufol" (Czechoslovakia) silica-gel plate. The radioactive zones of cholesterol and its esters formed as a result of biosynthesis and enzymatic hydrolysis were located on the chromatogram in a chloroform : methanol (95 : 5) system, placed in xylene scintillator and examined in the Mark-3 (USA) instrument. The error of measurement of enzyme activity was determined as the standard error of the rate of counting, with subsequent conversion of the deviation into experimental error by means of graphs showing counting rate as a function of quantity of labeled substance [1].

EXPERIMENTAL RESULTS

Control experiments on rat blood vessels showed that the process is enzymic in character, for the radioactivity of the reaction products sought increased depending on the incubation time, and also that the activity

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TABLE 1. ACAT Activity (substrate, [^{14}C]-cholesterol) in Intima of Human Aorta (in picomoles esterified cholesterol/mg protein·h; M ± m)

Age, years	Affected areas	Unaffected areas
37	3,8±0,1	2,7±0,1
44	3,6±0,1	8,6±0,1
46	4,0±0,1	0,7±0,1
46	0,5±0,1	0,0
46	11,4±0,1	3,2±0,1
46	1,0±0,0	0,3±0,1
52	9,9±0,1	2,2±0,1
58	8,0±0,2	1,4±0,1
58	26,9±0,3	8,5±0,2
60	9,0±0,2	8,2±0,2
60	0,4±0,0	0,0
63	25,6±0,3	9,8±0,2
64	1,2±0,0	19,5±0,1
65	9,0±0,1	15,4±0,1
66	7,3±0,2	3,1±0,1
68	2,2±0,0	22,8±0,2
72	5,7±0,2	11,9±0,2
80	1,8±0,0	0,2±0,0
80	0,2±0,0	1,0±0,1
85	1,2±0,1	4,8±0,1

TABLE 2. ACAT Activity in Human Aorta (typical lesions) (M ± m)

Type of lesion	Intima	Media	Adventitia
Fibrous plaque	0,47±0,11	0,92±0,14	0,51±0,11
Repetitive structure	2,2±0,18	—	—
Fibro-atheromatous plaque	1,27±0,15	1,03±0,15	1,75±0,18
Fibro-atheromatous plaque with ulceration	0,34±0,10	0,37±0,12	0,40±0,11
Unaffected area	1,20±0,15	0,30±0,10	1,30±0,17

Legend. Substrate and units of activity as in Table 1.

TABLE 3. Activity of Enzymes Synthesizing (substrate, palmityl-CoA) and Hydrolyzing (substrate, cholesteryl-palmitate) Cholesterol Esters in Intima of Children's Aortas (in picomoles esterified and hydrolyzed cholesterol/mg protein/h; M ± m)

Age	Synthesis	Hydrolysis
3 months	4403±39	4,2±0,3
2 years 4 months	2459±28	2,9±0,8
14 years	2903±30	4,4±0,4
17 years	2834±21	7,0±0,9
3.5 months	2524±12	5,0±0,8
5 years 10 months	4046±24	4,3±1,3
12 years	2373±7	3,4±0,3
13 years	3570±22	7,7±0,9

of the various enzymes studied did not change significantly during keeping of the cadavers of the animals for 3-21 h at 20°C compared with specimens from "fresh" blood vessels.

The results of determination of ACAT activity in the intima of the aorta from persons of different age groups are given in Table 1. Clearly there was no definite preponderance of ACAT activity in the affected areas over the intact regions. The distribution of ACAT activity in typical lesions of the aorta in different layers and in different types of lesions is shown in Table 2. Here also there was no significant quantitative difference in the synthesis of cholesterol esters in the affected and intact areas. This fact contradicts the data recalled above [4], but is confirmed by another investigation which showed that the biosynthesis of cholesterol esters in the aorta is directly dependent on the total quantity of cholesterol present at that given moment in the segment of aorta taken for analysis. Consequently, ACAT activity in a given area of the aorta will

be determined not only by the total quantity of cholesterol, but also by the quantity of ATP and of coenzyme A. This hypothesis was confirmed by measuring ACAT activity in different parts of the human aorta in the intima, media, and adventitia, using palmityl-[^{14}C]-CoA as the substrate. As Table 1 shows, ACAT activity in the human aorta, when labeled cholesterol was given, did not exceed 27 pmoles/mg protein·h, or 27 conventional units (c.u.). When labeled palmityl-CoA was used, ACAT activity was sharply "increased" and ranged from 250 to 400 c.u. in different parts of the intima, from 300 to 880 c.u. in the media, and between 100 and 970 c.u. in the adventitia. In parallel experiments, in the same areas activity of the enzyme hydrolyzing cholesterol esters was determined, using cholesteryl-palmitate as the substrate. The hydrolyzing activity varied from 1 to 3 c.u. in the intima, from 1.4 to 3.6 c.u. in the media, and from 2.0 to 4.6 c.u. in the adventitia. All the data given above relate to the adult human aorta. The aortas of children had appreciably higher ACAT activity than those of adults, although activity of cholesterol esterase in this case showed little change (Table 3).

The approach to the study of the role of enzymes synthesizing and hydrolyzing cholesterol esters in the processes of atherogenesis by seeking factors activating or inhibiting these enzymes, but without allowing for the whole range or, at least, the principal components which determine enzymic activity at a given moment, in the given area of the vessel wall, is thus technically incorrect.

LITERATURE CITED

1. C. Wang and D. Willis, Radioindicator Methods in Biology, Prentice-Hall (1965).
2. R. W. S. Clair, Ann. N. Y. Acad. Sci., 275, 228 (1976).
3. R. W. S. Clair, H. B. Lofland, and T. B. Clarkson, Circulat. Res., 27, 213 (1970).
4. S. Hachimoto, S. Daylon, R. B. Alfin-Slater, et al., Circulat. Res., 34, 176 (1974).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
6. R. J. Morin, E. E. Edralin, and J. M. Woo, Atherosclerosis, 20, 27 (1974).
7. A. H. Nilsson and F. L. Nordon, Biochim. Biophys. Acta, 296, 593 (1973).

MECHANISM OF METHYLATION OF DNA BASES BY SYMMETRICAL DIMETHYLHYDRAZINE

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The effect of disulfiram on alkylation of the purine bases of DNA in the liver and intestine of rats was studied during the action of 1,2-dimethylhydrazine-^{3}H (DMH). Preliminary administration of disulfiram, which inhibits N-oxidation of DMH, prevents methylation of the guanine of DNA. Under these circumstances, however, radioactivity is incorporated into normal (non-methylated) purine bases in the course of their synthesis. The absence of methylation of DNA during the action of disulfiram is evidence that it is alkylated by the carbonium ion and not by the methyl radical.

KEY WORDS: metabolism of 1,2-dimethylhydrazine; disulfiram; alkylation of DNA.

1,2-Dimethylhydrazine (DMH), which selectively induces intestinal neoplasms [3], leads to methylation of DNA in various tissues [1, 8, 12]. DMH metabolism begins with its dehydrogenation to azomethane [4]. The conversion of azomethane can take place either through N-oxidation with the formation of methylazoxymethane or by α-C-hydroxylation with conversion into methylazomethanol. In the first case, as a result of the decomposition of methylazoxymethanol a carbonium ion (CH_3^+) is formed, whereas in the second case it would be expected that a methyl radical ($C^{\bullet}H_3$) would be formed as a result of the homolytic breakdown of methyldiimine. However, these terminal particles, which differ in their chemical nature and reactivity, can interact differently

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