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Cholinesterase, Arylesterase, and Lipoprotein Parameters in Twins

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A high heritability estimate was obtained for serum arylesterase activity level in a series of 40 male twin pairs (23 MZ, 17 DZ) aged 33–39 years. The heritability estimated for cholinesterase activity was strikingly lower. In unrelated individuals a positive correlation between arylesterase, cholinesterase, and various lipoprotein parameters appeared to support the suggestion that these enzymes interact with various lipoproteins at the molecular level. The nature of these associations is not known. It is, however, tempting to speculate that some of the hereditary influence on lipoprotein levels in man may be mediated through the genetic control of one or both of these enzymes.

Key words: Heritability estimates, Cholinesterase, Arylesterase, Cholesterol, Triglycerides, Lipoproteins, Twins

INTRODUCTION

A variety of esterases are present in human tissues [21]. Several esterases are also found in human serum [3, 34]. Of these, cholinesterase (EC 3.1.1.8) and arylesterase (EC 3.1.1.2) are of particular interest, since their respective physiological functions remain obscure despite the fact that these enzymes are present with relatively high activity in the sera of all mammals [3].

Accumulating evidence suggests that both enzymes may be associated with different lipoprotein fractions [15, 5]. Several previous studies have indicated that the serum levels of both esterases in man may be under hereditary control [32, 33]. In the present study, the levels of both enzymes were determined in a series of twin pairs on whom data on several lipoprotein parameters were also available.

MATERIALS AND METHODS

Twins

The twins were drawn from the population-based Norwegian Twin Registry as part of an ongoing study [8]. A subsample had been randomly selected for genetic marker studies to validate the zygosity information obtained by questionnaire. All 40 pairs of male twins (23 MZ, 17 DZ) in this subsample who were 33–39 years old were included in the present study.

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Blood Sampling

Blood samples were drawn by the same physician on the morning after an overnight fast. A standardized procedure was used to reduce preanalytical variation. Serum free of visible hemolysis was stored at −20°C until used. To avoid the introduction of experimental biases, the samples were identified only by a random number until laboratory analyses had been completed.

Serum Cholinesterase Activity Determination

Butyrylthiocholine iodide (and in some experiments, also propionyl- and acetyl-thiocholine iodide from Sigma, St. Louis, MO) 2 mM in 50 mM Tris-HCl buffer pH 7.4 were used as substrate. The amount of free thiol groups hydrolyzed from the substrates and coupled to 0.12 mM DTNB (5,5′-dithiobis(2-nitrobenzoic acid)), was recorded photometrically at 410 nm after a fixed incubation time of 9 minutes at 25°C in a continuous flow system (AutoAnalyzer, Technicon Instruments Co., Tarrytown, NY). Serum samples were prediluted 1:500 with distilled water and entered through the sample line to a final concentration of 1:1,072. Serial dilutions in 1 mM EDTA of a 100-mM reduced glutathion stock solution were used as standards. All samples were analyzed in the same run. Coefficient of variation for the same serum independently diluted and analyzed ten times throughout the same run was less than 2%. The cholinesterase values were taken as the median value of three independently determined serum determinations. The cholinesterase inhibitor, R02-0683 (dimethylcarbamate of 2-hydroxy-5-phenylbenzyl trimethylammonium bromide) was obtained from Hoffmann–La Roche Inc., Nutley, NJ.

Serum Arylesterase Activity Determinations

Phenylacetate (Merck) in seven different concentrations (0.33–6.67 mM) was used as substrate in 50 mM Tris-acetate buffer at pH 7.5. The rate of hydrolysis at 25°C ± 0.1 was measured and the increase in the optical density at 269 nm recorded by a Beckman Model 35 Kinetic System. $CaCl_2$ was added to a final concentration of 1 mM by prediluting serum 1:101 with a $CaCl_2$ solution [22]. To start the reaction, 100 μl of this serum dilution were added to 2.0 ml preheated buffered substrate solution. The rate of hydrolysis was recorded for the initial 2–3 minutes. The observed linear change in optical density was converted to mmoles substrate hydrolyzed/second/liter serum (mKatal) by a conversion factor incorporating the molar absorbance of phenol experimentally determined under the conditions given.

All enzyme assays were carried out in triplicate and the median values selected for further analyses of data. A computer program calculating the best fit of the data to a hyperbolic function [16] yielded the maximum velocity (V_{max}) and the Michaelis constant (K_m). As a measurement of serum arylesterase activity, the velocity at 4 mM substrate concentration [22] is given when not specifically stated otherwise.

Measurements of Lipoprotein Parameters

Serum cholesterol and triglycerides were determined in a Multistat III Fast Analyzer (Instrumentation Laboratory, Lexington, MA) by the CHOD-PAP method and the fully enzymatic method (using lipase, esterase, GK, PK, and LDH), respectively, employing commercial reagent kits (Boehringer Mannheim GmbH, Mannheim, FRG). The levels of LDL and HDL (measured by content of major apoproteins, apoA-I and apoA-II) were determined by immunological methods [11].

Data Processing

The data were processed by a DEC-10 computer employing standard programs.

RESULTS

Reliability of the Enzyme Determinations

Under the specified experimental conditions, cholinesterase inhibitors (eserine 10 μM, R02-0683 1 μM) inhibited the cholinesterase activity, but had no effect on the arylesterase activity. Conversely, arylesterase but not cholinesterase activity, was strongly dependent on the calcium ion concentration of the assay solution. Without the addition of calcium,

arylesterase was inhibited by lanthanium chloride (50 μM) and by EDTA (200 μM), whereas these salts exhibited no inhibitory effect on cholinesterase determinations. Thus, the two assay procedures used apparently measured two different enzyme functions.

Cholinesterase activity was also determined with acetyl- and proprionyl-thiocholine as substrates. These results were in complete agreement ($r \geqslant 0.99$) with those obtained with butyrylthiocholine, which is the substrate of choice [31]. At 2 mM substrate concentration, the activity levels of cholinesterase on proprionyl- and acetyl-thiocholine were 95% and 47% of that on butyrylthiocholine, respectively.

The dilutions of serum used for the enzyme assays (1:1,072 and 1:2,121 for cholinesterase and for arylesterase, respectively) made the employment of a two-step dilution procedure desirable. Serum for cholinesterase activity measurements was prediluted in water. The diluted sera exhibited the same activity after storage for one week at 4°C, and 80% activity when left for another week at room temperature. In contrast, arylesterase activity, although stable to storage in serum [22], immediately decreased when serum was diluted with water or buffer. This deterioration was retarded when serum was diluted in the $CaCl_2$ solution. The arylesterase activity remained stable for the duration of the experiments in the present study (less than 30 minutes per sample) only when such diluted samples were kept in an ice bath.

Distribution of the Enzyme Parameters

The enzyme activities observed and the kinetic parameters calculated, were approximately normally distributed (Table 1), allowing the use of parametric statistical tests. Two samples were identified through incomplete inhibition by R02-0683 to have the intermediate cholinesterase phenotype (genotype $E_1^a E_1^u$). These samples were drawn from each of the two members of a DZ twin pair. Analyses of data after the exclusion of this pair gave the same results as analyses of the total body of data. No attempts were made to identify other possible genetic variants of cholinesterase loci [32].

Heritability Estimates of Cholinesterase and Arylesterase

The results of the various heritability analyses are given in Table 2. The results of F tests indicated that no corrections of the heritability estimates were required [14]. A strong indication of hereditary influence on the observed enzyme levels was obtained only in the case of arylesterase. Similar analyses carried out for tests also employing phenylacetate concentrations of 1 and 2.5 mM, resulted in h^2 estimates for arylesterase activity of 0.80 and 1.00, respectively. Hereditary influence on the cholinesterase activity could not be excluded. An estimate of heritability close to zero was found for the Michaelis constant.

TABLE 1. Distribution of Serum Enzyme Activities in the Twin Subjects (n = 80)

	Mean	SD	Median	Skewness
Cholinesterase[a]	96.5	22.9	98.0	0.007
Arylesterase[a,b]	1.81	0.42	1.77	0.005
V_{max} arylesterase[a]	2.26	0.53	2.16	0.005
K_m arylesterase[c]	1.02	0.18	1.02	0.004

[a]mKatal l^{-1}.
[b]Substrate concentration, 4 mM.
[c]mM.

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Association Studies Between Various Parameters

For association studies, only results from unrelated individuals were used (Table 3). Cholinesterase levels were found to be positively correlated with arylesterase activity, but the two enzymes exhibited individual associations to the liproprotein parameters. Thus, the level of cholinesterase (but not of arylesterase) was positively correlated to cholesterol level and possibly to triglyceride levels. On the other hand, arylesterase was strikingly correlated to both the HDL-apoproteins A-I and A-II.

DISCUSSION

The present data indicated that arylesterase activity, and to some extent cholinesterase activity in serum, may in part be determined by genetic factors. Other investigators have found a considerably higher hereditary influence on cholinesterase levels [see 20], whereas the present arylesterase heritability estimates were in agreement with the findings of Simpson [33]. The present data were based on analysis of samples from male twin pairs of similar age. Thus, complex corrections for the influence of age and sex on enzyme activities [4, 22, 30, 33] were unnecessary, but the results are valid only for adult males.

For both enzymes, molecular heterogeneity has been claimed. Such a heterogeneity could possibly interfere with the present analyses, since only serum total enzyme activities were determined. The multiple electrophoretic components of cholinesterase, which by genetic evidence have been shown to be the product of at least two structural loci [19], most likely owe their esterase activity to the function of only one common structural locus [25, 26]. The cholinesterase activity measured by the present technique did not follow simple Michaelis-Menten kinetics. Recent studies have documented that the non-hyperbolic kinetics of cholinesterase nevertheless should be interpreted in terms of a single enzyme [18].

The molecular heterogeneity claimed to exist in arylesterase [3, 13] has been challenged [12, 22]. In the present study, human serum arylesterase followed simple Michaelis-Menten kinetics, and evidence for the involvement of more than one enzyme was not found.

The arylesterase levels observed in each serum did not show a significant correlation with individually calculated Michaelis constants ($r = 0.031$). The observed variations in these constants between sera were not significantly influenced by hereditary factors (Table 2). Thus, it was concluded that the observed hereditary influence on serum arylesterase level was not determined through genetically determined differences in the arylesterase molecular properties (as reflected by the K_m) but rather in the variations in the serum concentration of the same enzyme (as reflected by V_{max}).

In the present study, a positive correlation between the activities of cholinesterase and arylesterase in serum was detected. Such a correlation was not found in a group of 74 patients in a mental hospital [24]. However, the cholinesterase of patients in a mental hospital may not be considered to be qualitatively nor quantitatively normal [35, 29]. Furthermore, the enzyme assay conditions employed in the present study were different from those used previously [24]. It may be worthwhile to test for this possible association between the two major serum esterases in a larger series of unrelated healthy individuals.

Butyrylthiocholine and phenylacetate are usually considered to be hydrolyzed only by cholinesterase and by arylesterase, respectively [3]. The two enzymes measured reacted in their characteristic way [3, 21] towards their respective inhibitors. Also, the marked difference observed in their respective stability following serum dilution indicated that two

TABLE 2. Analysis of Variance and Heritability Estimates ($h^2 = 2(r_{MZ} - r_{DZ})$) *for Serum Cholinesterase and Arylesterase Activities*

		Mean	SD	Var_{betw}	Var_{with}	r_{intrap}	h^2
Cholinesterase[a]	MZ	99.2	21.9	923.68	56.76	0.88	0.18
	DZ	92.8	23.9	1,049.38	123.85	0.79	
Arylesterase[a,b]	MZ	1.86	0.45	39.04	2.36	0.89	0.72
	DZ	1.74	0.37	20.82	6.36	0.53	
V_{max} arylesterase[a]	MZ	2.33	0.56	61.15	3.60	0.89	0.64
	DZ	2.17	0.47	34.74	9.68	0.57	
K_m arylesterase[c]	MZ	1.03	0.19	6.38	1.28	0.66	0.08
	DZ	1.00	0.17	4.57	1.06	0.62	

[a]mKatal l^{-1}.
[b]Substrate concentration, 4 mM.
[c]mM.

TABLE 3. Correlation Coefficients Between Cholinesterase and Arylesterase Activities and Various Lipoprotein Parameters in Unrelated Individuals

	Cholinesterase	Cholesterol	Triglyceride	LDL	ApoA-I	ApoA-II
Cholinesterase	–	0.380* (0.364)	0.299 (0.109)	0.134 (0.119)	0.044 (0.086)	0.235 (0.251)
Arylesterase	0.362* (0.550)	0.093 (0.162)	−0.072 (−0.195)	−0.104 (0.041)	0.497** (0.413)	0.637** (0.317)

The values are drawn from a subsample composed of one twin from each pair (H = 40) drawn at random. The values from the co-twin subsample are shown in brackets for comparison.
*$P < 0.05$.
**$P < 0.01$.

different enzymes were measured. Moreover, a sample from a patient homozygous for the rare silent cholinesterase gene (patient IV-2 from [2]), which had no cholinesterase activity, exhibited normal arylesterase activity. On the basis of these observations, it was considered unlikely that the observed correlation between the two enzyme levels should be the result of insufficiently specific laboratory procedures. Rather, a biological explanation should be sought.

The two enzyme levels showed different and significant positive associations to various serum lipoprotein parameters (Table 3). The most apparent correlations were found between serum total cholesterol and cholinesterase, and between the major apoproteins of HDL and arylesterase levels. Since variations in lipoprotein levels in man are influenced by hereditary factors [6], it will be important to establish whether some of this hereditary influence may be mediated through genetic control of serum cholinesterase or of arylesterase levels. However, it cannot be excluded that serum cholinesterase levels may chiefly be determined by lipoprotein levels [17], whereas the high heritability estimates observed for arylesterase levels may favor a more direct genetic influence on the serum level of this enzyme.

Several previous reports have demonstrated a possible physiological role in lipid metabolism of cholinesterase [see 23] and of arylesterase [27]. However, neither of these reports prove a primary effect of these enzymes in the determination of normal serum lipid levels in man.

Arylesterase was intimately associated with the HDL apoproteins A- I and A-II (Table 3). This class of lipoproteins has recently received great interest for its possibly protective role against coronary heart disease, and low HDL levels may be seen among relatives of patients suffering from this disease [28]. It remains to be seen whether arylesterase is involved in this effect.

Serum arylesterase levels are also in other species known to be controlled by genetic factors [33]. The laboratory rabbit may be of particular interest for model studies on the relationship between arylesterase and HDL, as genetic markers are known both in arylesterase [1] and in HDL [7, 9, 10].

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