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THE RECOGNITION OF ATYPICAL PLASMA CHOLINESTERASE BY RELATIVE SUBSTRATE HYDROLYSIS RATE*

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SUMMARY

An improved method for the identification of persons heterozygous or homozygous for the dibucaine-resistant form of atypical plasma cholinesterase (acylcholine acyl-hydrolase, EC 3.1.1.8) has been developed. It relies on the spectrophotometric determination of the ratio of the rates of hydrolysis of 50 μM procaine and tetracaine. Discrimination between normal and abnormal homozygotes and heterozygotes is clearer with this method than with the conventional dibucaine number. The results obtained with 74 individuals of verified genotype are given.

INTRODUCTION

The existence of a qualitatively distinct form of cholinesterase (acylcholine-acyl-hydrolase, EC 3.1.1.8) in the plasma of persons who experienced prolonged apnea following the administration of succinyldicholine was established by Kalow (ref. 1; see also review in ref. 2). Subsequently, Kalow and Staron[3] demonstrated that the percentage inhibition of the cholinesterase-catalyzed hydrolysis of 50 μM benzoylcholine produced by 10 μM dibucaine (dibucaine number, DN)[4] had a trimodal distribution. Persons exhibiting marked sensitivity to succinylcholine were usually homozygous for atypical plasma cholinesterase (DD) and had a low percentage inhibition by dibucaine (low DN)[5]. Individuals homozygous for the normal enzyme (NN) had a high DN while heterozygotes for the normal and atypical enzymes (ND) showed intermediate DN values.

Davies *et al.*[6] compared the hydrolysis rates of a series of acetylcholine homologues by normal and atypical plasma. The differences in relative hydrolysis rates of the various substrates encouraged Rubinstein and Deitz[7] to employ the ratio of

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hydrolysis of benzoylcholine and acetylcholine for the identification of the three phenotypes. While both the DN and the ratio of benzoylcholine to acetylcholine hydrolysis rates are quite satisfactory for the identification of DD individuals, a clear differentiation between NN and ND persons is not always possible with these methods[8,9].

Preliminary studies with NN and DD plasma revealed that, of a large number of substrates studied, the relative hydrolysis rate (DD/NN) of procaine was the lowest and that of tetracaine the highest. The relative hydrolysis rates for benzoylcholine, acetylcholine, procaine and tetracaine determined on ten pooled NN and ten pooled DD plasmas are given in Table I. It was postulated that determination of the ratio of the hydrolysis rates of procaine and tetracaine might allow a better discrimination between the three genotypes than that provided by the other two methods.

TABLE I

HYDROLYSIS RATES OF VARIOUS SUBSTRATES BY POOLED NORMAL AND ATYPICAL HUMAN PLASMA CHOLINESTERASE

Substrate	*Hydrolysis rate**		*Ratio (atypical/normal)*
	Normal homozygote	*Atypical homozygote*	
Acetylcholine	236	47	0.20
Benzoylcholine	83	31	0.37
Procaine	1.1	0.18	0.16
Tetracaine	0.20	0.23	1.15

* μmoles of substrate hydrolyzed per ml plasma per h.

MATERIALS AND METHODS

Plasma was obtained from persons who had experienced prolonged apnea following the administration of succinylcholine. In those cases where the presence of atypical cholinesterase was confirmed by the DN, plasma samples were also obtained from relatives of the propositi. The 19 persons who were phenotypically atypical homozygotes had all experienced succinylcholine-induced prolonged apnea themselves or were relatives of such persons. It was possible to show from family studies of DN values that 14 of these were DD homozygotes and that 5 were DS heterozygotes, possessing one silent cholinesterase gene (S) of the type described originally by Liddell *et al.*[9]. Persons whose genotype could not be determined from family studies were not included in this series. All but one of the 30 ND heterozygotes were relatives of propositi who had experienced apnea. The exception was a person who experienced a short period of apnea following administration of succinylcholine during electroshock therapy. The 25 phenotypically normal persons were also relatives of the propositi. Five of these could be shown by family studies to be NS heterozygotes. The remaining 20 persons belonged to families in which no silent gene could be demonstrated and they were assigned to the NN genotype.

In each case heparinized blood was obtained and the plasma was separated and stored at −20 °C. Acetylcholine hydrolysis was determined by a null-point potentiometric procedure using the pH-Stat (Radiometer, Copenhagen), essentially by the

method of Smith *et al.*[10]. The substrate concentration was 20 mM and the assays were carried out at 37 °C. The hydrolysis rates of the aromatic substrates (50 μM concentration) were determined with a Beckman DU spectrophotometer at 37 °C. The following wavelengths (in nm) were used to follow the hydrolysis: benzoylcholine, 246; procaine, 290; tetracaine, 313. For benzoylcholine hydrolysis rates a final assay dilution of plasma of 1:200 was employed. For procaine and tetracaine the plasma dilution was 1:20. Dibucaine numbers were determined by the method of Kalow and Genest[4]. The units of all the hydrolysis rates are μmoles of substrate hydrolyzed per ml of plasma per h.

RESULTS AND DISCUSSION

The hydrolysis rates of acetylcholine, benzoylcholine, procaine and tetracaine of the 74 individuals studied are presented in Table II together with DN, benzoylcholine/acetylcholine ratios and procaine/tetracaine ratios. The attributions of genotype to members of Groups II–V (NS, ND, DD, DS) are certain on the basis of family studies, as are those of the majority of Group I (NN). However, it is possible that one or more members of Group I could be NS.

TABLE II

THE HYDROLYSIS RATES OF ACETYLCHOLINE, BENZOYLCHOLINE, PROCAINE, AND TETRACAINE AND THE DIBUCAINE NUMBERS (DN), BENZOYLCHOLINE–ACETYLCHOLINE (B/A) AND PROCAINE–TETRACAINE (P/T) HYDROLYSIS RATE RATIOS FOR 74 INDIVIDUALS OF FIVE GENOTYPES

	Acetylcholine	*Benzoylcholine*	*Procaine*	*Tetracaine*	*DN*	*B/A*	*P/T*
Group I (Genotype NN)							
1	339	121	1.80	0.44	70	0.36	409
2	304	108	1.54	0.40	72	0.36	385
3	310	100	1.62	0.40	70	0.32	405
4	225	70	0.60	0.15	73	0.31	400
5	353	109	1.62	0.50	67	0.31	324
6	260	97	1.41	0.47	72	0.37	300
7	273	87	1.40	0.38	72	0.32	368
8	230	78	1.05	0.34	70	0.34	309
9	331	92	1.20	0.33	74	0.28	364
10	328	116	1.54	0.41	72	0.35	376
11	299	86	1.38	0.34	73	0.29	406
12	197	68	1.17	0.32	72	0.35	366
13	285	73	1.11	0.32	73	0.26	347
14	298	86	1.07	0.32	73	0.29	334
15	249	83	1.14	0.33	73	0.33	345
16	287	105	1.48	0.36	73	0.37	411
17	170	68	0.88	0.23	69	0.40	383
18	238	97	1.25	0.39	73	0.41	321
19	310	117	1.36	0.39	73	0.38	349
20	315	121	1.61	0.43	72	0.38	374
Group II (Genotype NS)							
1	121	43	0.58	0.14	69	0.35	420
2	166	51	0.78	0.23	70	0.31	345
3	223	62	0.90	0.26	71	0.28	347
4	110	47	0.65	0.17	66	0.43	373
5	154	65	0.90	0.27	68	0.42	341

TABLE II *(continued)*

	Acetylcholine	*Benzoylcholine*	*Procaine*	*Tetracaine*	*DN*	*B/A*	*P/T*
Group III (Genotype ND)							
1	152	48	0.69	0.31	53	0.32	223
2	115	62	0.98	0.38	58	0.54	258
3	176	63	0.93	0.38	59	0.36	245
4	199	67	1.04	0.41	59	0.34	254
5	197	72	0.81	0.42	60	0.37	193
6	153	58	0.69	0.34	55	0.38	203
7	93	42	0.42	0.22	46	0.45	191
8	206	72	0.86	0.42	62	0.35	205
9	163	58	0.65	0.36	54	0.36	181
10	312	117	1.48	0.64	60	0.38	231
11	202	91	0.94	0.44	55	0.45	214
12	101	52	0.55	0.31	52	0.51	177
13	237	90	1.03	0.44	63	0.38	234
14	126	51	0.60	0.40	50	0.40	150
15	103	40	0.40	0.24	57	0.39	167
16	125	61	0.64	0.38	50	0.49	168
17	128	59	0.56	0.40	46	0.46	140
18	109	51	0.45	0.24	53	0.47	188
19	186	80	0.95	0.44	58	0.43	216
20	121	53	0.62	0.26	58	0.44	238
21	264	100	1.12	0.49	61	0.38	229
22	170	73	0.75	0.41	53	0.43	183
23	123	66	0.61	0.33	53	0.54	185
24	98	36	0.51	0.23	61	0.37	222
25	173	62	0.86	0.40	56	0.36	215
26	333	82	1.17	0.54	58	0.25	217
27	105	39	0.43	0.26	53	0.37	165
28	134	51	0.71	0.32	61	0.38	229
29	105	43	0.47	0.31	50	0.41	152
30	100	46	0.56	0.25	61	0.46	224
Group IV (Genotype DD)							
1	42	34	0.16	0.38	13	0.81	42
2	38	29	0.20	0.38	17	0.76	53
3	49	31	0.19	0.41	18	0.63	46
4	56	37	0.25	0.50	16	0.66	50
5	69	48	0.26	0.52	16	0.70	50
6	87	54	0.20	0.54	14	0.62	37
7	80	48	0.31	0.52	7	0.60	60
8	62	43	0.20	0.43	8	0.69	47
9	30	28	0.09	0.36	6	0.93	25
10	71	40	0.27	0.55	10	0.56	49
11	44	26	0.10	0.37	8	0.59	27
12	37	25	0.17	0.34	9	0.68	50
13	48	33	0.13	0.37	10	0.69	35
14	39	25	0.15	0.44	11	0.64	34
Group V (Genotype DS)							
1	31	20	0.16	0.26	10	0.65	62
2	30	21	0.13	0.26	12	0.70	50
3	39	23	0.15	0.28	11	0.59	54
4	26	18	0.12	0.22	8	0.69	54
5	13	12	0.03	0.15	14	0.92	22

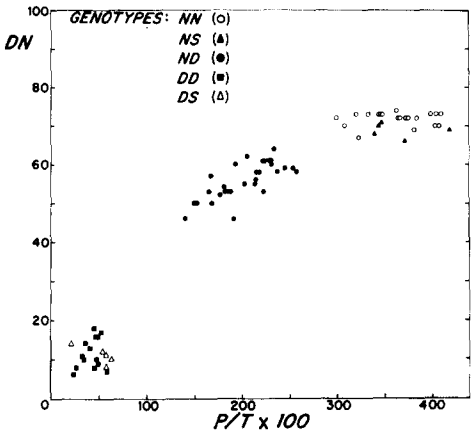

Fig. 1. The ratio of the hydrolysis rates of procaine and tetracaine by the plasma of 74 individuals of various genotypes plotted against the dibucaine numbers. The genotypes are represented by the following symbols: ○, NN; ▲, NS; ●, ND; □, DD; △, DS.

Fig. 1 shows a plot of the DN and procaine/tetracaine ratio for each member of the series. As the actual ratio contains decimal fractions, for convenience each value is multiplied by 100. Throughout this paper the term procaine/tetracaine ratio is to be understood as containing this factor of 100. No distinction can be made between the NN and NS groups, nor between the DD and DS groups in terms of procaine/tetracaine ratio. There is a suggestion that the mean DN for NS persons may be slightly lower than for NN, an observation confirmed in a larger series of NS persons not included in this study. However, the hydrolysis rates of the various substrates by plasma from subjects who had one S gene were significantly depressed. This is evident in Table III, which presents the mean values of the seven variables for each of the five genetic groups. The values for the hydrolysis rates of the four substrates for the NS and DS groups varied between 52% and 62% of those for the corresponding homozygotes. The mean fall of the NS group was to 57% of the NN group and that of the DS group to 55% of the DD group. As SS homozygotes show no cholinesterase activity

TABLE III

MEAN VALUES OF THE HYDROLYSIS RATES OF ACETYLCHOLINE, BENZOYLCHOLINE, PROCAINE AND TETRACAINE AND DIBUCAINE NUMBERS (DN) BENZOYLCHOLINE–ACETYLCHOLINE (B/A) AND PROCAINE–TETRACAINE (P/T) HYDROLYSIS RATE RATIOS FOR FIVE GROUPS OF GENOTYPICALLY DISTINCT INDIVIDUALS

Each value is the arithmetic mean ± the standard error of the mean.

Substrates	*Group I NN (N = 20)*	*Group II NS (N = 5)*	*Group III ND (N = 30)*	*Group IV DD (N = 14)*	*Group V DS (N = 5)*
Acetylcholine	280 ± 11	155 ± 22	160 ± 12	53.7 ± 4.9	27.8 ± 3.4
Benzoylcholine	94.1 ± 4.0	53.6 ± 4.8	62.8 ± 3.6	35.8 ± 2.9	18.8 ± 2.1
Procaine	1.31 ± 0.07	0.762 ± 0.073	0.749 ± 0.048	0.191 ± 0.018	0.118 ± 0.08
Tetracaine	0.363 ± 0.018	0.214 ± 0.028	0.365 ± 0.018	0.436 ± 0.021	0.234 ± 0.026
DN	71.8 ± 0.4	68.8 ± 1.0	55.8 ± 0.9	11.6 ± 1.1	11.0 ± 1.1
B/A	0.34 ± 0.01	0.36 ± 0.06	0.41 ± 0.01	0.68 ± 0.03	0.71 ± 0.05
P/T	364 ± 7.7	365 ± 16.6	203 ± 5.9	43.2 ± 2.8	51.8 ± 8.4

TABLE IV

GENE EXPRESSIVITY IN THE ND, NS AND DS HETEROZYGOTES FOR FOUR SUBSTRATES USING THE DATA OF TABLE III

Genotype	*Substrate*	$b/(a+c)$*	*Mean* $b/(a+c)$
ND	Acetylcholine	0.479	0.480
	Benzoylcholine	0.483	
	Procaine	0.499	
	Tetracaine	0.457	
NS	Acetylcholine	0.554	0.550
	Benzoylcholine	0.570	
	Procaine	0.582	
	Tetracaine	0.590	
DS	Acetylcholine	0.518	0.550
	Benzoylcholine	0.525	
	Procaine	0.618	
	Tetracaine	0.537	

* Gene expressivity, where a, b and c represent the mean values for normal homozygotes, heterozygotes and abnormal homozygotes, respectively (see text).

detectable by conventional methods, it is unlikely that the extra activity is due to the S gene product. It is possible that there is compensatory production of N or D gene product when the S gene is present.

The principal finding, however, is, as predicted from the preliminary data of Table I, that the procaine/tetracaine ratio provides the maximum discrimination between the three phenotypes studied. The procaine/tetracaine ratio does indeed allow an unambiguous differentiation between the three phenotypes superior to that provided by the DN. This is most clearly shown in Fig. 1 where the separation between the NN and ND groups is considerably wider in terms of procaine/tetracaine ratio than for DN. Thus, for family studies in our laboratory the procaine/tetracaine ratio has largely replaced determination of DN. Both methods require two spectrophotometric assays of each plasma and can be carried out in approximately the same time. Although the hydrolysis rates of procaine and tetracaine are considerably lower than that of benzoylcholine, this is compensated for by decreasing the dilution of plasma employed in the assay and by a much larger absorbance change on hydrolysis (benzoylcholine 0.75, procaine 3.2, and tetracaine 4.5 absorbance units per mole, approximately).

The investigation of families carried out in the course of this study revealed that 5 of the 19 individuals who had a DN or procaine/tetracaine ratio in the atypical homozygote range were genotypically DS. Since these individuals are in separate families, this allows an approximate gene frequency for the silent cholinesterase gene to be calculated. The gene frequency for the D gene is given by Kalow and Gunn[11] as 0.01884, from which the frequency of DD homozygotes in a healthy Canadian population was calculated to be 0.000355 (1:2820). From our data the frequency of DS heterozygote is 26% of this value, or 0.0000810 (1:11 000). The frequency of the S gene is thus $0.0000910 \div 0.01884 \div 2 = 0.00242$. The frequency of NS heterozygotes is then 0.48% (1:200) and of SS homozygotes 0.00000586 (1:170 000). These figures are similar to those calculated by Motulsky[12] and Goedde and Altland[13].

The data on the hydrolysis rates of the four substrates presented in Table III

allows calculation of the gene expressivity of the cholinesterase genes involved. The results are presented in Table IV, using the formula of Harris[14] where the gene expressivity $= b/(a + c)$, where a, b and c represent the mean values found for normal homozygotes, heterozygotes and abnormal homozygotes, respectively. The N and D genes are equally expressed in the ND heterozygote with a value of 0.48, little different from the figure of 0.52 given by Harris (see ref. 12). It appears that the N gene in the NS heterozygote and the D gene in the DS heterozygote show a greater contribution than expected, to the extent of 15% and 10%, respectively.

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