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UDPglucuronosyltransferase of the human placenta

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THE PLACENTA of many species including man exhibits drug metabolizing activity.[1–4] Of the second phase, i.e. conjugative detoxication reactions, acetylation has been found to take place in rabbit placenta,[5] and sulfate conjugation in guinea pig[6] and human placenta.[7] UDPglucuronosyltransferase activity, responsible for glucuronide formation, has been detected in the placenta of the rabbit,[5,8] rat and guinea-pig,[8] but not in man.[9,10] Because of the availability of a sensitive method, used to determine UDPglucuronosyl transferase activity in placentae from other species, and other tissues with low enzyme activity,[8] the activity in the human placenta was reinvestigated.

Thirty-seven placentae were collected in spontaneous deliveries, and three in Caesarean sections. A piece of the placenta was immediately removed, and cooled in ice-cold 0·25 M sucrose. Within 30 min, the piece was weighed, homogenized in 4 vol of the isotonic sucrose with a Potter–Elvehjem glass-Teflon homogenizer, and centrifuged at 12,000 g_{max} for 10 min at 0° in a Sorval RC2B centrifuge. The supernatant fraction, unless immediately used, was stored at 0° until the next day. A microsomal fraction was prepared using the calcium aggregation method described earlier[8] and activated with digitonin.[11] Microsomes from 200 mg wet tissue were used to determine UDPglucuronosyltransferase (UDPglucuronate β-glucuronosyltransferase (acceptor unspecific, E C 2.4.1.17) activity, and 4-methylumbelliferone was used as the aglycone. Twenty-five microlitres of microsomal suspension was added to 100 μl of a 0·1 M phosphate buffer (pH 7·0) fortified with 1·6 mM UDPglucuronic acid (Sigma Chemical Company, St. Louis, Mo., U.S.A.) and 0·1 mM 4-methylumbelliferone (British Drug Houses, Ltd., Poole, England). The reaction was stopped after 30 min by the addition of 5 ml of chloroform and 5 ml of 0·05 M acetate buffer (pH 5·0). After extraction with chloroform (5 ml three times), 0·5 ml of the aqueous phase was added to 1·5 ml of 0·01 M acetate buffer (pH 5·0) containing 100 units of β-glucuronidase (0·1 mg, type b1 from bovine liver, Sigma). After 30 min at 37°, 1·0 ml of 1·6 M glycine buffer, pH 10·35 was added and the fluorescence was measured with an Aminco-Bowman spectrofluorometer using 370 nm as the activating and 450 nm as the emitting wavelength. Incubations without added UDPglucuronic acid served as the blanks, the values of which were subtracted from the actual determinations. In some instances, 10 mM D-glucaro-1,4-lactone was included in the β-glucuronidase hydrolysis

The weight of the placentas was 518 $\pm$ 113 g (S.D.); the protein content of the microsomal fraction was 0·78 $\pm$ 0·13 mg/g (S.D.). The UDPglucuronosyltransferase activity of 16 out of the 40 placentas studied exceeded 20 pmoles/min/g wet wt. This limit represented a fluorescence twice that in the blanks (S.D. of the blanks was 15 per cent) and has been regarded as the limit of the sensitivity of the method. The highest activity detected was 327 pmoles/min/g (Table 1).

Because the synthesis of the conjugate was dependent on the presence of UDPglucuronic acid, the conjugate formed was hydrolyzed by β-glucuronidase, and this hydrolysis was quantitatively inhibited by 10 mM D-glucaro-1,4-lactone, a specific inhibitor of β-glucuronidase,[12] it is evident that glucuronide synthesis really was concerned. The failure of Chakraborty *et al.*[9] to demonstrate UDPglucuronosyltransferase (*p*-nitrophenol) activity in the human placenta apparently was due to the insensitivity of the method: even the most sensitive method using *p*-nitrophenol, which uses radiolabeled *p*-nitrophenol,[13] although 100 times more sensitive than the standard method, is less sensitive than the method used in the present study, which is capable of detecting the synthesis of about 10 pmoles of the glucuronide. The controversy of the present results with those of Kyecombe *et al.*,[10] who used 4-methylumbelliferone as the aglycone, might be explained by (1) the use of digitonin to activate the enzyme, (2) the use of a large amount of microsomes, (3) destruction of the enzyme during storage (up to 90 per cent in 4 hr), or (4) differences in the patient material; in the present study, 60 per cent of the placentas were devoid of UDPglucuronosyltransferase activity.

The remarkable differences in the UDPglucuronosyltransferase activity in various placentas were not due to use of different parts of the placenta, because no significant variation could be detected in microsomes from different parts of a single placenta. The loss of activity during storage (0°) of the 12,000 *g* supernatant fraction was less than 10 per cent in 24 hr. Specific inhibition of β-glucuronidase by D-glucaro-1,4-lactone (cf. 12) as described[8] did not increase the measurable UDPglucuronosyltransferase activity. Evidently the high pH value in the determination of β-glucuronidase as well as the unfavourable kinetic circumstances with a high concentration of the reaction product (4-methylumbelliferone) and quite a low concentration of the substrate (4-methylumbelliferylglucuronide) inhibit β- glucuronidase action. Variations in UDPglucuronosyltransferase activity therefore are not due to variations in β-glucuronidase activity. The highest activities were found in placentae from nonsmokers; thus cigarette smoking, known to

TABLE 1. UDPGLUCURONOSYLTRANSFERASE (4-METHYLUMBELLIFERONE) ACTIVITY OF HUMAN PLACENTAL MICROSOMES AND SMOKING HABITS OF THE MOTHERS

No. of patient	4-Methylumbelliferone conjugated (pmoles/min/g wet wt)	Cigarettes/day
1	0	0
2	0	0
3	0	?
4	0	?
5	1	?†
6	2	0
7	2	0
8	3	10–15
9	3	0
10	4	0
11	4	?
12	4	0
13	5	10
14	5	0
15	6	0
16	6	20
17	7	0
18	8	0
19	8	10
20	8	0
21	11	10
22	12	0
23	16	0
24	19	0
25	21*	0
26	22	0
27	23	0
28	23	0
29	43	?†
30	44	15
31	44	0
32	46	10
33	54	?†
34	58	0
35	66	0
36	69	?
37	106	0
38	136	0
39	160	0
40	327	0

* Values above 20 pmoles/min/g are regarded as significantly different from zero.

† Caesarean section.

enhance the hydroxylation activity of human placenta,[3,14–17] seems to be without effect on UDPglucuronosyltransferase activity. These two functionally closely linked activities seem to vary independently.

The great variation in the activity of the UDPglucuronosyltransferase resembles that of human placental hydroxylation;[18] the clinical significance of this finding remains to be elucidated in a further study.

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Inhibition of monoamine oxidase activity by propargylamine in pituitary cells in culture: lack of effect on cell growth or prolactin production*

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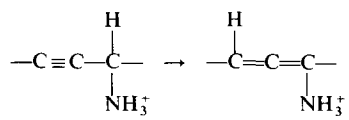
A NUMBER of acetylenic compounds, for instance pargyline, inhibit monoamine oxidase (MAO) activity.[1] It has been proposed that the basis of the inhibition is the ability of these compounds to rearrange to allenes upon interaction with the active site of the enzyme.[2] The allene then reacts with functional groups at the active site



to bind covalently and inactivate the enzyme. Accordingly, the simplest compound which should inhibit MAO is propargylamine ($CH \equiv C—CH_2NH_2$). We have found that propargylamine inactivates and covalently labels mitochondrial monoamine oxidase† and plasma amine oxidase *in vitro*.[2] The compound also

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