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PRELIMINARY COMMUNICATION

DIAZEPAM METABOLISM IN HUMAN CORTEX KIDNEY MICROSOMES

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Recently, a cytochrome P-450 linked monooxygenase system and its hydroxylating activity towards lauric acid has been demonstrated in human kidney cortex microsomes [1]. In the present communication the metabolism in human kidney of diazepam, a drug widely used for treatment of emotional disorders, is described.

Material and methods

The metabolic studies were performed with cortex of kidneys taken at operation from three patients for medical reasons (table 1). The homogenization of apparently healthy parts of the parenchyma was performed with an Ultra Turrax and the microsomal fraction was isolated according to Ernster et al.[2]. The microsomal suspension was stored at - 15°C in a freezer until use within two weeks. The protein concentration was determined by the lowry method [3]. Cytochrome P-450 and b_5 were estimated according to Jacobsson and Cinti [1]. The difference spectra were recorded with an Unicam Sp 800 coupled with a Beckman recorder to expand the spectrum. NADPH-cytochrome c reductase was measured as described by Philipps and Langdon [4].
The incubation of the microsomal suspension was performed as described previously [5]. After incubation the vessels were stored in a freezer at - 15°C until analysis by gas chromatography [5].

Results and discussion

The specific activities of the NADPH-cytochrome c reductase in human kidney cortex microsomes were extremely low as compared with those in human adult and fetal liver microsomes. Cytochrome P-450 was not detected in the microsomal fraction. The values for cytochrome b_5

are given in table 1.

Table 1

Diagnoses of the patients studied and the specific activities of NADPH-cytochrome c reductase (cyt. c red.) and cytochrome b_5

Pat.	age (yrs.)	sex	diagnosis	cyt.c reduct^X cyt.c reduced nmol./mg x min^{-1}	cyt.b_5 nmol./mg	P-450
W.W.	72	m.	kidney cortex adenoma	15	0.23	n.d.
M.S.	65	f.	hypernephroid carcinoma	10	0.11	n.d.
G.B.	24	m.	kidney rupture	8.3	0.14	n.st.

n.d.: not detectable; n.st.: not studied (the material was too small for spectral analysis); X values found in human adult liver microsomes : 126 nmoles[6] and in human fetal liver microsomes : 47 nmoles[7].

In the three kidneys studied the metabolic pathway of diazepam (D) is shown to involve C 3-hydroxylation and N_1-demethylation yielding N-methyloxazepem (MO) and N-desmethyldiazepam (DD) (table 2).

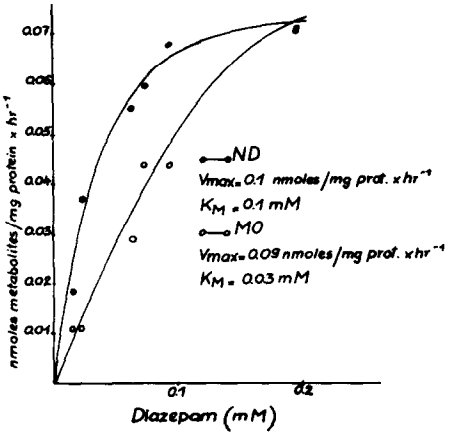

Fig. 1 Rate of biotransformation in human kidney cortex microsomes from pat. W.W. as a function of diazepam concentration

Kidney cortex microsomes from patient W.W. were incubated with different concentrations of D. The hyperbolic curves for both metabolic products are depicted in fig. 1. The V_{max} values calculated by the direct linear plot according to Eisenthal and Cornish-Bowden[8] were 0.1 nmoles / mg protein x hr^{-1} and 0.09 nmoles / mg protein x hr^{-1} for MO and DD, respectively. It should be noticed that the reaction velocity was found to be linear up to 20 minutes incubation time using human liver microsomes[5]. Therefore, the actual V_{max} values are probably somewhat higher than 1.7 and 1.5 pmoles / mg protein x min^{-1}.

Table 2

Diazepam metabolizing activity in kidney cortex microsomes

Pat.	D	MO	DD
	mM	nmoles/mg prot.x hr^{-1}	
G.B.	1.0	1.0	0.2
	0.1	0.14	0.06
W.W.	1.0	0.45	0.1
	0.1	0.08	0.08
M.S.[x]	1.0	+	+

x Reliable estimates of the quantities of metabolites formed were not possible with the analytical method used in this study.

On the basis of these data, it is suggested that human kidney cortex microsomes can degrade D although the kidneys studied are shown to be deficient in cytochrome P-450. It should be emphasized that this microsomal pigment was measured by a spectrophotometric equipment probably not sensitive enough to detect traces of cytochrome P-450. The diazepam metabolizing capacity of human kidney is considerably lower than that of human liver and therefore seems to be without any significance on the plasma elimination rate of D. The possibility exists

that activity may rise under certain conditions, thus increasing the proportion of D metabolized by kidney.

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