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INDIVIDUAL DIFFERENT RESPONSE TO DRUGS: CHARACTERISATION OF AN INH-ACETYLATING SYSTEM IN RHESUS MONKEYS*†

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Abstract—The biochemical properties of an INH acetylating enzyme protein of liver homogenates from rhesus monkeys were studied. The enzyme was purified about 250-fold. Studies of substrate specificity to aliphatic and aromatic compounds as well as comparison of the structure of some competitive inhibitors were performed.

Serotonin is acetylated to acetyl serotonin with high affinity to the INH acetylating enzyme. Enzyme preparations from so-called rapid and slow acetylating animals show different specific activities, but similar properties regarding purification values, K_m-values of INH, inhibitor constants of some aromatic compounds. Comparing arylamine acetylase of pigeon liver with the acetylating system of rhesus-liver, we stated characteristic differences.

THE DRUG isonicotinic acid hydrazide (INH) which is utilized in the therapy of tuberculosis is converted into different inactive metabolites in human beings.[1, 2] After family studies, an interindividual different conversion of INH, a high intraindividual constancy, and high concordancy in identical twins, resp., was observed.[3–5] A genetically determined polymorphism of INH-inactivation was suggested.[6] A large amount of applicated INH is transferred to acetyl-INH;[7] according to,[6, 2, 8] an acetylation mechanism was suggested as the cause of this polymorphism.

To study the biochemical properties of acetylating enzyme proteins in correlation with a rapid or slow inactivation of INH, we studied the metabolism of the latter in monkeys.[9, 10] It was proved that the transfer of the acetyl-group of acetyl coenzyme A proceeds towards INH via an INH-acetylating system; we used alternatively ^{14}C-acetyl coenzyme A or ^{14}C-labelled INH. Our parallel investigations of the different INH-acetylation *in vivo* and *in vitro* showed a good conformity of results. To check qualitative differences in acetylase proteins, a spectrophotometric test according to Jenne *et al.*[11] was modified[9]: Instead of acetyl coenzyme A the model substance N,S-diacetylcysteamine was used as an acetyl-group donor.[12] Reaction assay of our standard system: INH $5{\cdot}8 \times 10^{-3}$ M, N, S-diacetylcysteamine $1{\cdot}9 \times 10^{-3}$ M, sodium diphosphate $8{\cdot}5 \times 10^{-2}$ M, 0·1 ml enzyme solution, volume 3 ml, 1-cm cell, 25°, 303 mμ, pH 9·0.[13]

The sp. act. (standard system; ΔE/min/mg protein) of enzymes of 110 rhesus

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monkeys (*Macaca mulatta*) show a multimodal distribution very similar to that of human beings (INH plasma level),[14] but the frequency of rapid or slow INH converting individuals differ strongly in man and monkey.[15]

To characterize the INH-acetylating system the enzyme was purified from liver of *M. mulatta* about 250-fold after acetone precipitations by fractionating on DEAE-cellulose (buffer: Tris-hydroxy-methyl-aminomethane 10^{-2}M, elution: NaCl-gradient 0·018/0·18 M, pH 8·0). Using purified enzyme preparations, we compared the acetylating proteins of so-called rapid and slow INH-inactivating animals and studied, if there might be similarities to the acetylating system of pigeon liver (arylamine-acetylase). The average value of the INH-conversion per mg protein of a slow acetylating animal is about 23 mμM/min.; after purification of the enzyme, this value increases up to 5700 mμM/min.

The unstable purified enzymes can be stabilized by addition of sodium thioglycolate (10^{-4} M) for 24 hr. The crude liver homogenate from rhesus monkeys is stable for 3 hr at 37°; there is a decrease of activity of 40 per cent in 5 min at 45°; in 5 min at 50° there is a 100 per cent inactivation.[13, 16] There are no differences in enzyme activity in the range of pH 7·5–9·5; a specific pH-optimum must be investigated with higher purified enzyme preparations.

Regarding substrate specificity, we could show that aliphatic amines, which are usually acetylated in the organism, as glucosamine[17], carnitine,[18] choline,[19] glutamic acid, and others,[20, 21] have no effect of inhibition with the INH-acetylating enzyme up to concentration of $1{\cdot}6 \times 10^{-2}$ M. However, the aromatic compounds, we examined (see Table 1), showed a different mode of inhibition in correlation with structural and functional groups. The physiological active compounds serotonin and tryptamine show the highest affinity to the INH-acetylating system (see Table 1, part A); other aromatic amines as tyramine (4), phenyl-ethyl-amine (5), and histamine (6) show less affinity (see Table 1, part A). We are now investigating the possibility that these acetylases might be important in the metabolism of serotonin or tryptophane. We could prove by radio-thinlayer-chromatography techniques that serotonin is acetylated to acetyl serotonin.[15]

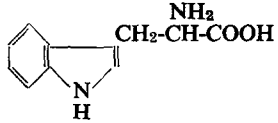
Comparing the structure of the competitive inhibitors (see Table 1, part A) and the corresponding K_i-values, we observed that in group I the decarboxylation of tryptophane is followed by increasing affinity (factor 10^2; 1, 2), whereas an additional hydroxy-group in the indole component causes no change in affinity (2, 3). Substitution of the indole-group by benzene nucleus (3, 4), is correlated with decreasing affinity. Similar to division (I), in division (II) the existence of a hydroxy-group belonging to the benzene ring does not change affinity (4, 5). However, after a substitution of the benzene nucleus by the imidazol-group (5, 6), there is a 5–6-fold decrease in affinity; substitution by the indole-group (5, 2) is followed by increase of affinity of about 10 times. In column (III) the transformation of the carboxylic group into its amide (7, 8) causes a 3-fold increase of inhibition. Of interest is the comparison of *p*-aminobenzoate (13), *p*-aminosalicylate (14), *p*-nitroaniline (12), and sulfanilamide, resp. (15): We could not observe any affinity of the INH-acetylating system to *p*-aminobenzoate (13); a substitution of the COOH-group by the NO_2-group does not change the affinity (13, 12). A combination of a hydroxy-group and a COOH-group in ortho-position (14) causes a non-competitive inhibition. The substitution of the carboxy-group of *p*-aminobenzoate by a sulfonamide residue (13, 15) causes competitive inhibition in

TABLE 1. CHARACTERISTICS OF THE INH-ACETYLATING SYSTEM

(A) K_i-values of the competitive inhibitors of the INH-acetylating system of rapid and slow acetylating rhesus monkeys.

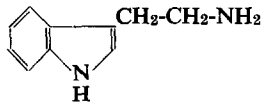
(I)

(1) L-Tryptophane



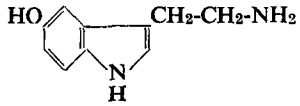
K_i $1{\cdot}8 \times 10^{-2}$ M/l

(2) Tryptamine



K_i $1{\cdot}0 \times 10^{-4}$ M/l

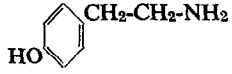
(3) Serotonin



K_i $1{\cdot}1 \times 10^{-4}$ M/l

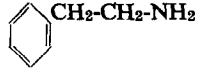
(II)

(4)Tyramine



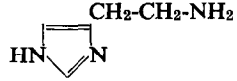
K_i $1{\cdot}2 \times 10^{-3}$ M/l

(5) Phenylethylamine



K_i $1{\cdot}5 \times 10^{-3}$ M/l

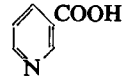
(6) Histamine



K_i $8{\cdot}5 \times 10^{-3}$ M/l

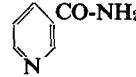
(III)

(7) Nicotinic acid



K_i $1{\cdot}0 \times 10^{-2}$ M/l

(8) Nicotinamide



K_i $3{\cdot}0 \times 10^{-3}$ M/l

TABLE 1—*continued*

(B) K_m- and K_i-values of various substrates and inhibitors of the acetylating enzymes of rhesus monkeys and pigeons.

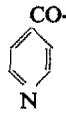
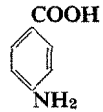
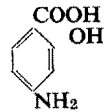
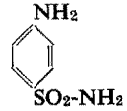
No.	Substrate	Structural formula	Conversion of substrates rhesus	Conversion of substrates pigeon	K_m-values (mole/l). rhesus	K_m-values (mole/l). pigeon
(9)	Acetyl coenzyme A	$H_3C\text{-}CO\text{-}SCoA$	+	+	$4{\cdot}6 \times 10^{-4}$	$4{\cdot}5 \times 10^{-4}$
(10)	N,S-diacetyl-cysteamine	$H_3C\text{-}CO\text{-}S(CH_2)_2\text{-}N(H)\text{-}CO\text{-}CH_3$	+	+	$4{\cdot}6 \times 10^{-4}$	300×10^{-4}
(11)	Isonicotinic acid hydrazide		+	+	27×10^{-4}	$0{\cdot}8 \times 10^{-4}$
(12)	*p*-Nitroaniline		−	+	—	$0{\cdot}6 \times 10^{-4}$
	Inhibitor				K_i-values (mole/l). [Type of inhibition]	
(13)	*p*-Aminobenzoate		−	+	28×10^{-3} (*non-competit.*)	$0{\cdot}83 \times 10^{-3}$ (competitive)
(14)	*p*-Aminosalicylate		−	+	$5{\cdot}0 \times 10^{-3}$ (non-competit.)	$0{\cdot}93 \times 10^{-3}$ (competitive)
(15)	Sulfanilamide		+	+	$3{\cdot}2 \times 10^{-3}$ (competitive)	— (competitive)

TABLE 1—*continued*

(C) Apparent Michaelis constants for N,S-diacetylcysteamine and INH (rhesus monkeys).

Co-reactant N,S-diacetylcysteamine	K_m INH (Mole/l)	Co-reactant INH	K_m N,S-diacetylcysteamine (mole/l)
$9{\cdot}3 \times 10^{-5}$ M	45×10^{-5}	12×10^{-5} M	97×10^{-5}
$27{\cdot}9 \times 10^{-5}$ M	100×10^{-5}	20×10^{-5} M	194×10^{-5}
$186{\cdot}0 \times 10^{-5}$ M	250×10^{-5}	45×10^{-5} M	582×10^{-5}

the same range as that of *p*-aminosalicylate. The K_i-values of the investigated inhibitors are very high in aromatic systems with a carboxylic-group (low affinity) (1, 7) or show very small affinity like *p*-aminobenzoate (13); but the hydroxy-group besides the COOH-group causes a greatly increased affinity (*p*-aminosalicylate, 14). The hydroxy-group without a carboxylic-group is not of importance for the kinetic constants (2/3, 4/5). Measuring the K_m-values of acetyl-coenzyme A and N,S-diacetylcysteamine we stated, that the affinity of the INH-acetylating system is similar regarding the mentioned acetyl-donators (see Table 1, part B/9, 10). The K_m-values are correlated to the concentration of the co-reactant (see Table 1, part C). The K_m-value increases with increasing concentration of the other reactant. We determined the K_m-values by the method of Dixon (s/v towards s).[22] Regarding INH and N,S-diacetylcysteamine, there is an inhibition by excessive concentrations of the respective substrates.

Enzyme preparations of different specific activities of rapid and slow acetylating animals have similar properties: Factor of purification by acetone-fractionations, K_m-values of INH and N,S-diacetylcysteamine, inhibitor constants of serotonin, tryptamine, tyramine, phenylethylamine, nicotinic acid, and sulfanilamide (see Table 1 part A). These results are interpreted as a hint, that the suggested qualitative differences of the acetylating systems of rhesus monkeys are very small, corresponding to similar results in man.[11] It might be possible to investigate the qualitative differences of the enzyme proteins, using very highly purified preparations.

However, comparing the arylamine acetylase of pigeon liver and the acetylating system of rhesus-liver (Table 1, part B), we stated characteristic differences. There are the same K_m-values of both enzymes regarding to acetyl coenzyme A (INH-concentration $5{\cdot}8 \times 10^{-3}$ M); however, regarding to the K_m-values of the model substance N,S-diacetylcysteamine (with an INH-concentration of $5{\cdot}8 \times 10^{-3}$ M), and otherwise to the K_m-values of INH as substrate (with a N,S-diacetylcysteamine concentration of $1{\cdot}9 \times 10^{-3}$ M), there are differences with the factor 100.

While the arylamine acetylase of pigeon liver acetylates *p*-nitroaniline with high affinity to this substrate (12) (see Table 1, part B), there is no acetylating effect by liver homogenates from rhesus monkey. The components (13), (14), (15) cause a competitive inhibition of the arylamine acetylase; regarding the INH-acetylating system, there is a competitive inhibition (15), non-competitive inhibition (14), or nearly no inhibition (13).

Both enzymes are inhibited by excessive substrate concentrations of INH and N,S-diacetylcysteamine. Besides this, both enzymes are characterized by the fact, that there is a dependence of the K_m-values regarding INH and N,S-diacetylcysteamine to increasing concentrations of the co-reactant (see above, and Table 1, part C). This fact was also observed by Jenne and Boyer[11] in 1962, investigating the arylamine acetylase reaction with acetyl coenzyme A.

Further investigations of the interesting acetylating system may clarify the cause of the differing specific activities in rapid and slow INH-acetylating animals.

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