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The effect of antidepressants on ethylmorphine and imipramine *N*-demethylation in rat liver microsomes

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The effects of single and multiple doses of desipramine, amitriptyline or citalopram on the rat liver microsomal cytochrome P-450 level and on the rate of ethylmorphine and imipramine demethylation in-vitro have been investigated. Desipramine, amitriptyline or citalopram when given to rats as a single dose, did not affect the level of cytochrome P-450 in the liver microsomes, however, there was a tendency towards acceleration of imipramine, and particularly ethylmorphine, demethylation. Prolonged administration of desipramine and citalopram, but not amitriptyline, elevated the microsomal level of cytochrome P-450 and accelerated the rate of ethylmorphine demethylation. All the drugs investigated, when given chronically, inhibited the rate of imipramine demethylation. Since demethylation of ethylmorphine and imipramine in a CO atmosphere was inhibited by ca 90% for the former and only by 58% for the latter, it can be assumed that prolonged administration of the drugs investigated has two different effects on the oxygenase systems in rat liver microsomes: on the one hand they stimulate the cytochrome P450 oxygenase system involved in ethylmorphine demethylation and, on the other, they inhibit the other microsomal oxygenase system involved in demethylation of imipramine.

Antidepressants (AD) are often used in pharmacological experiments and in therapy together with other drugs whose biological effects may be potentiated or inhibited by them. It has been found that prolonged administration of imipramine and desipramine enhances the level of cytochrome P450 in rat liver microsomes (Breyer 1972; Daniel et al 1984), accelerates the rate of ethylmorphine and perazine demethylation in in-vitro experiments (Breyer 1972), and stimulates $^{14}CO_2$ exhalation after administration of [*N*-methyl-^{14}C]-benzphetamine to rats (Daniel et al 1984). Therefore a biological interaction between imipramine and other drugs might be induced, at least partially, by metabolic alterations.

To find whether other AD, like imipramine, exert similar effects on the activity of metabolizing enzymes, we investigated the effect of single and multiple doses of desipramine, amitriptyline and citalopram (an antidepressant still undergoing clinical trial) on the liver microsomal cytochrome P450 level and on the rate of ethylmorphine and imipramine demethylation in-vitro.

The main step of AD biotransformation in rat liver microsomes is *N*-demethylation which, according to Bickel (1971), Gigon & Bickel (1971) and Nakazawa (1970), is catalysed by microsomal enzymes via the cytochrome P450 system and proceeds by an α-*C*-oxidation mechanism. However, another pathway of imipramine and other AD *N*-demethylation is also possible, i.e. *N*-oxidation, the mechanism of which is still controversial.

As the results of our study indicated some discrepancies between the changes in cytochrome P450 level in rat liver microsomes and imipramine demethylase activity, we also attempted to compare the mechanism of ethylmorphine and imipramine demethylation.

Methods

The experiments were on male Wistar rats (200–250 g) kept under standard laboratory conditions and fed a standard granulated diet (Bacutil) with free access to tap water.

Imipramine hydrochloride (Polfa, Starogard), desipramine hydrochloride (Serva, Heidelberg), amitriptyline hydrochloride (Natterman, Köln), citalopram hydrobromide (Lundbeck, Copenhagen), diethylmorphine (hydrochloride, Merck), NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase (Boehringer, Mannheim) were used.

Rats received desipramine, amitriptyline or citalopram in a single intraperitoneal dose of 10 mg kg^{-1}, or were treated for 14 days with the same dose, i.e. 10 mg kg^{-1}, of the drugs at 24 h intervals. The animals were decapitated 24 h after AD administration. Control animals received 0·9% NaCl (saline). Each group consisted of 5 rats.

Livers were excised and microsomes prepared according to conventional methods by a differential centrifugation in Tris/KCl buffer (pH = 7·4). Cytochrome P450 concentration was determined according to Omura & Sato (1964) as a reduced carbon monoxide complex in a UV-Vis-SPECORD spectrophotometer. Protein was assayed according to Lowry et al (1951), with bovine serum albumin as a standard.

The incubation mixture contained: microsomal protein 2 mg ml^{-1}, ethylmorphine or imipramine 0·3 μM, NADP 0·1, nicotinamide 30, glucose-6-phosphate 1·2, magnesium chloride 2·5, Tris-KCl 105 mM, pH = 7·4 + 0·3 u ml^{-1} glucose-6-phosphate dehydrogenase. The final incubation volume was 1 ml. The mixture was incubated in a water bath at 37 °C for 30 min. Each sample had its own control. The control for the saline-treated rats was prepared as above using liver microsomes from the same animals but without the addition of substrate (ethylmorphine or imipramine).

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Table 1. The effect of a single, 10 mg kg^{-1} i.p. dose of AD (desipramine DMI, amitriptyline AMI, citalopram CIT) on the level of liver microsomal cytochrome P450 and formaldehyde formation from ethylmorphine and imipramine. The animals were killed 24 h after AD administration. Results are means of 5 determinations ± s.e.m.

Drugs	Relative liver weight (%)	Cytochrome P450 level (nmol (mg protein)$^{-1}$)	% of control	Formaldehyde (nmol (mg protein)$^{-1}$/ 30 min) from ethylmorphine	% of control	Formaldehyde (nmol mg protein)$^{-1}$/ 30 min) from imipramine	% of control
Saline	4·23 ± 0·05	0·563 ± 0·02	—	27·90 ± 1·77	—	33·05 ± 1·74	—
DMI	4·41 ± 0·10	0·578 ± 0·02	102·58	40·75 ± 4·80	146·03	39·24 ± 4·86	118·72
AMI	4·13 ± 0·16	0·569 ± 0·01	101·13	41·56 ± 3·27	148·93	38·59 ± 3·23	116·74
CIT	4·22 ± 0·13	0·565 ± 0·03	100·4	40·00 ± 6·77	143·35	42·73 ± 2·08	143·35

Table 2. The effect of prolonged administration of AD in a dose of 10 mg kg^{-1} i.p. for 14 days on the relative liver weight, cytochrome P450 level and formaldehyde formation from ethylmorphine and imipramine. Rats were killed 24 h after the last dose of AD. Results are means of 5 determinations ± s.e.m. Statistical significance $^{*}P < 0·05$; $^{**}P < 0·01$ when compared with saline-treated animals (control group) (Duncan's test).

Drugs	Relative liver weight (%)	Cytochrome P450 level (nmol (mg protein)$^{-1}$)	% of control	Formaldehyde (nmol (mg protein)$^{-1}$/ 30 min) from ethylmorphine	% of control	Formaldehyde (nmol (mg protein)$^{-1}$/ 30 min) from imipramine	% of control
Saline	3·41 ± 0·15	0·489 ± 0·02	—	31·66 ± 1·33	—	36·95 ± 2·42	—
DMI	4·20 ± 0·30	0·598 ± 0·00**	120·35	40·93 ± 1·79*	129·25	29·42 ± 2·53	79·6
AMI	3·68 ± 0·13	0·475 ± 0·02	97·21	29·15 ± 2·35	92·06	28·22 ± 3·16	76·3
CIT	3·76 ± 0·39	0·575 ± 0·01*	117·29	38·33 ± 2·58*	121·07	26·60 ± 2·87	72·0

Table 3. Ethylmorphine and imipramine demethylation in-vitro in the CO atmosphere. Data are the mean ± s.e.m. from 5 rats. * Indicates significant difference from control values: $P < 0·001$ (Student's t-test).

Substrate	Formaldehyde formation (nmol (mg protein)$^{-1}$/30 min) Control	CO	% K
Ethyl-morphine	45·30 ± 0·39	4·70 ± 0·62*	10·37
Imipramine	28·88 ± 0·60	12·14 ± 1·15*	42·85

The control for AD-treated animals was similarly prepared, i.e. using the liver microsomes from AD-treated rats without substrate. The reaction was terminated with 0·1 ml of 60% perchloric acid. The amount of formaldehyde from ethylmorphine or imipramine was determined according to Nash (1953) and was calculated from the difference between the sample reading and the reading of the corresponding control. There was no difference beween readings of saline control and AD-treated control.

Cytochrome P450 inactivation was with carbon monoxide (approx. 70% CO and 30% air) during incubation.

The results were evaluated statistically using an analysis of variance followed by Duncan's test, and Student's t-test.

Results

Administration of a single, 10 mg kg^{-1} dose of desipramine, amitriptyline or citalopram did not affect the level of cytochrome P450 in rat liver microsomes, however, it stimulated formaldehyde formation from both ethylmorphine and imipramine (Table 1).

Prolonged administration of desipramine and citalopram to rats produced an elevation of cytochrome P450 (approx. by 20%) in rat liver microsomes. Amitriptyline did not affect the concentration of cytochrome P450. Repeated treatment with desipramine and citalopram enhanced the rate of ethylmorphine demethylation, while the rate of imipramine demethylation was decreased by all the AD (Table 2).

Demethylation of ethylmorphine or imipramine, in the presence of CO, produced inhibition of ethylmorphine demethylation by approximately 90%, while imipramine demethylation was inhibited by some 58% (Table 3).

Discussion

As indicated by our previous results (Daniel et al 1984) as well as those presented herein, changes induced by prolonged administration of AD are not limited to biochemical and receptor alterations in the central nervous system of rats (Vetulani et al 1976; Smith et al 1981), but also occur at the level of metabolizing enzymes. Desipramine, citalopram and imipramine (Daniel et al 1984), when given to rats for two weeks, enhanced the level of liver microsomal cytochrome P450 in a statistically significant manner. Amitriptyline did not affect the cytochrome P450 level.

The AD tested affected the rate of demethylation of ethylmorphine and imipramine. A single dose of AD accelerated the rate of formaldehyde formation from

ethylmorphine and imipramine. It is difficult to assume that the overall effect is due to a real enzymatic induction, as it requires a prolonged administration of the inducer to animals (Testa & Jenner 1976). A two-week treatment with desipramine and citalopram elevated the rate of demethylation of ethylmorphine, which corresponded well with the enhanced cytochrome P450 level induced by prolonged administration of AD. It was also in agreement with the earlier results of Kato et al (1965) which indicated that ethylmorphine demethylation proceeds via the oxygenase system involving liver microsomal cytochrome P450. Amitriptyline, which did not affect the cytochrome P450 level, also did not influence the rate of ethylmorphine demethylation.

Some discrepancies arise when the effect of the AD on imipramine demethylase activity is compared with their effect on the cytochrome P450 level and ethylmorphine demethylation. According to Bickel (1971), and Gigon & Bickel (1971), demethylation of imipramine proceeds via an α-*C*-oxidation mechanism and is catalysed by the cytochrome P450 system; therefore acceleration of imipramine demethylation can be suspected. Our results indicate that despite the enhancement of cytochrome P450 level by prolonged administration of AD the rate of imipramine demethylation is decreased. Interestingly, amitriptyline, which did not alter the cytochrome P450 level in liver microsomes of rats, also showed a tendency to inhibit formaldehyde formation from imipramine. Since in our experiment demethylation of ethylmorphine and imipramine in a CO atmosphere was inhibited by approximately 90% for ethylmorphine and 58% for imipramine, it can be assumed that imipramine demethylation proceeded, at least partially, via the oxygenase system, independent of cytochrome P450.

The data presented indicate that AD given to rats for two weeks induce the cytochrome P450 oxygenase system responsible for ethylmorphine demethylation. Since the rate of imipramine demethylation was inhibited after prolonged administration of AD, it seems that AD exert two different effects on the oxygenase systems in rat liver microsomes: on the one hand they stimualte the cytochrome P450 oxygenase system involved in ethylmorphine demethylation and, on the other, they inhibit the other microsomal oxygenase system involved in demethylation of imipramine. Therefore it is possible that the discrepancies found in the mechanism of demethylation of tertiary amines (Sugiura et al 1977) may be due to changes in the relation between the activities of some oxygenase systems involved in *N*-demethylation. These changes may be induced by different environmental conditions, such as diet, etc.

The above data may also have a more general meaning. When AD are used together with other drugs for a longer period, their effects on the activity of metabolic enzymes should be recalled since these effects may either stimulate or inhibit the biotransformation of other substances and, in consequence, produce an increase or decrease in the pharmacological response, or even evoke toxicity.

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