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Effect of imipramine on hepatic gamma-glutamyltransferase in female rats. Interaction with contraceptives

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The increase of gamma-glutamyltransferase (GGT, EC 2.3.2.2) in the plasma of treated subjects has been reported for numerous drugs, as described in a recent review [1]. Besides the measurement of GGT activity in clinical chemistry in cases of liver injuries, particularly in cholestasis, carcinoma, and after alcohol consumption, the use of this enzyme as an indicator of induction by certain drugs, especially those of the phenobarbital group, has been proposed [2]. Correlations between induction of liver drug-metabolizing enzymes and GGT in the liver have not been verified for all inducers.

We have recently shown that liver GGT is localized mainly in plasma membranes [3] and that its passage into the plasma after phenobarbital treatment is related to membrane disturbances due to induction and to solubilizing effect of bile salts [4, 5]. We have also found elevated GGT in the plasma of subjects treated long-term with imipramine. This elevation was particularly important in women who were also taking oral contraceptives [6].

In order to clarify the role of membrane alterations in the phenomenon of the release of GGT into the plasma after administration of such drugs, this paper reports a study of variations of GGT in the rat under the influence of imipramine and steroid contraceptives. These two drugs are known to have effects on liver membranes and, in some cases, their hepatic toxicity can lead to intrahepatic cholestasis [7, 8]. Furthermore, interactions of imipramine and oral contraceptives, which were manifested in some cases by mental disturbances, were extensively studied in females of different species [9]. It is not yet clearly established how these phenomena could be related to interaction in hepatic metabolism of the two drugs. We have studied, therefore, the variations of GGT and cytochrome P-450 (cyt. P-450) in the liver and plasma of female rats treated with imipramine, Mestranol–Norethynodrel mixture (MNm), or both administered simultaneously.

The effect of imipramine and MNm (obtained from Sigma Chemical Co, St. Louis, MO, U.S.A.) on the hepatic GGT activity was determined in liver subcellular fractions of female Sprague–Dawley rats (160–180 g body wt). The animals were divided into four groups: a control group received daily 0.3 ml of corn oil; the imipramine group was injected with a saline solution of imipramine (20 mg·kg^{-1}·day^{-1}); the contraceptive-treated group was injected with 0.3 ml of a corn oil solution containing Mestranol (0.07 mg·kg^{-1}·day^{-1}) and Norethynodrel (4 mg·kg^{-1}·day^{-1}); and the last group received injections of both imipramine solution and Mestranol–Norethynodrel mixture. The drugs were injected intraperitoneally over a period of 15 days.

The rats were fasted 16 hr before being killed. Plasma membranes were prepared from livers as described by Neville [10]. When complete subfractionation was performed, we used the method of Amar-Costesec *et al.* [11]. Proteins were assayed by the method of Lowry *et al.* [12] and GGT according to the method of Szasz [13] at 37° using L-gamma-glutamyl-3-carboxy-4-nitroanilide as the substrate. The concentration of cyt. P-450 was determined in microsomes by the method of Matsubara *et al.* [14]. Serum alkaline phosphatase (ALP, EC 3.1.3.1) was assayed at 30° by the method of Morgenstern *et al.* [15] and alanine aminotransferase (ALT, EC 2.6.1.2) by a kinetic optimized method at 30° using a reagent kit from Boehringer. Statistical differences were compared between control and treated groups using comparison of variances test.

Since it is known that GGT is localized in plasma membranes, in the first part of the experiments we investigated its variations under different treatments only in this fraction. But it was very surprising that the activities found in the plasma membranes did not reflect those measured in the homogenate when the rats received imipramine either alone or associated with MNm (Table 1). Indeed, the ratio of specific activity of GGT in plasma membranes to that in homogenate was 26 in controls and 23 in MNm-treated rats, but when imipramine or a combination of imipramine and MNm were administered, this ratio decreased to 4.4 or 4.6, respectively. These variations were due to the loss of GGT in plasma membranes under the effect of imipramine. Indeed, administration of imipramine did not alter significantly total activity in the liver homogenate (607 mU/g wet liver against 672 mU/g in controls) but resulted in an important decrease in the enzyme activity in the plasma membranes. In the same way, the combined treatment of rats with imipramine and MNm led to a 5-fold increase of GGT in the liver homogenate but did not change the plasma membrane activity. Conversely, there was a 2-fold increase in GGT activity in both homogenate and plasma membranes when the rats received MNm only. These data indicate that when imipramine was administered either alone or combined with MNm the subcellular distribution of GGT was altered. To understand how this phenomenon occurred, we studied the variations of the

Table 1. Effect of treatment with imipramine and Mestranol–Norethynodrel mixture on liver plasma membrane GGT in female rats

Treatment (N)*	Homogenate		Plasma membranes		Plasma membranes (mU/mg proteins)
	mU/g wet liver	mU/mg proteins	mU/g wet liver	mU/mg proteins	Homogenate (mU/mg proteins)
None (5)	672 ± 40	3.62 ± 0.52	122.0 ± 14	94.8 ± 20.5	26.0
Imipramine (5)	607 ± 105	3.39 ± 0.87	31.4 ± 12†	14.8 ± 3.1†	4.4†
MNm (5)‡	2066 ± 580†	8.50 ± 2.59†	280.0 ± 95†	198.0 ± 45.8†	23.0
Imipramine (5) + MNm‡	3256 ± 827†	13.75 ± 4.65†	114.0 ± 62	63.5 ± 26.5	4.6†

* Number of experimental animals used. The results are the means ± S.D. of N experiments.
† $P < 0.01$. Significantly different from control.
‡ Mestranol–Norethynodrel mixture.

Table 2. Effect of treatment with imipramine and Mestranol–Norethynodrel mixture on liver subcellular distribution of GGT in female rats

Treatment (N)*	Homogenate	Nuclei	Mitochondria–lysosomes	Microsomes	Cytosol
None (8)	656 ± 30	374 ± 44	44 ± 5	224 ± 27	23 ± 4
Imipramine (6)	570 ± 26	274 ± 35‡	33 ± 10	174 ± 34‡	67 ± 6†
MNm (4)§	2676 ± 649†	1943 ± 427†	101 ± 40‡	530 ± 147†	71 ± 14†
Imipramine (4) + MNm§	3171 ± 420†	1921 ± 243†	246 ± 36†	640 ± 226†	169 ± 20†

* Number of experimental animals used. The values, expressed as mU/g wet liver, are the means ± S.D. from N experiments.
† $P < 0.01$. Significantly different from control.
‡ $P < 0.05$. Significantly different from control.
§ Mestranol–Norethynodrel mixture.

enzyme distribution in the other subcellular fractions, as indicated in Table 2. The complete fractionation of liver cells showed that in the control livers the main activity of GGT was found in the fractions containing plasma membranes, such as the nuclear fraction (plasma membranes precipitate with nuclei during ultracentrifugation in the technique of fractionation) and microsomes (contaminated by plasma membranes), whereas the lysosomal–mitochondrial fraction and the cytosol showed very low activity. In the MNm-treated rats GGT activity was increased in the nuclei, microsomes and cytosol by 5.0-. 2.4- and 3-fold, respectively, when compared with the controls. Imipramine treatment resulted in a decrease in GGT activity in nuclei and microsomes and an increase in the cytosol (3-fold higher than in controls). Finally, when the animals were treated with the two drugs together, a remarkable increase in the enzyme activity of the cytosoluble fraction was observed (7.4-fold greater than the control activity). Our results indicate that under the influence of imipramine the enzyme activity lost from plasma membranes was found in the cytosol. Furthermore, Table 3 shows that in the plasma, the values of GGT activity were increased 3-, 3- or 7-fold in the rats treated with imipramine, MNm or the combination of the two compounds, respectively, when compared to the controls. These results are in agreement with previous findings in man concerning the increase of GGT in plasma of subjects under long-term treatment with imipramine and particularly of women receiving oral contraceptives at the same time [6].

It seems that the increase of GGT activity in the cytosol and plasma and its decrease in liver membranes are due to fragilization of membranes under the effect of imipramine which ensues the enzyme solubilization. This phenomenon could be explained by the high accumulation of imipramine in the membrane structures [16].

Variations of ALP activity in the plasma under the various treatments were not significant in comparison with those of GGT, which appears to be more sensitive in reflecting the effects of such treatment on hepatic cell membranes. Furthermore, these drugs did not cause liver 'cytolysis' because ALT activities in the plasma were not altered.

On the other hand, the variations of GGT activities in our experiments show that the influence of treatment with contraceptive steroids (MNm) in increasing the level of enzyme in the liver indicate an inducing effect of this drug. However, the effect of oral contraceptives on drug metabolism is controversial: impairment of drug metabolism capacity has been reported in a large group of women taking oral contraceptives [17]; but also other reports present evidence of the increase of drug-metabolizing rate in women taking oral contraceptives [18]. In fact, it seems that oral contraceptives are drug metabolic inhibitors for low doses and in the short term but inducers for high doses and chronic usage [19], which could explain the conflicting results found by different authors. Under our experimental conditions and at our dosage levels, the increase of GGT under MNm treatment was compatible with an inducing effect of these contraceptive steroids.

This phenomenon was amplified when imipramine was added to the MNm treatment. This increase of the response to MNm caused by imipramine is also pointed out by the

Table 3. Variations of hepatic parameters in female rat plasma under imipramine and Mestranol–Norethynodrel mixture treatment

Treatment (N)*	GGT	ALP	ALT
None (13)	1.40 ± 0.15	72 ± 27	46.6 ± 11.6
Imipramine (10)	4.07 ± 0.26†	62 ± 12	42.0 ± 5.2
MNm** (8)	3.94 ± 0.71†	79 ± 14	53.1 ± 5.9
Imipramine (8) + MNm**	10.00 ± 2.45†	93 ± 10	36.4 ± 5.2

* Number of experimental animals used. The results are expressed as mU/ml (GGT: gammaglutamyltransferase, ALP: alkaline phosphatase, ALT: alanine aminotransferase). The values are the means ± S.D. from N experimental animals.
† $P < 0.01$. Significantly different from control.
‡ Mestranol–Norethynodrel mixture.

uterotropic stimulation in rats [20] and in the incidence of neurological problems in women taking the two drugs [9]. These interferences probably proceed from imipramine inhibition of contraceptive metabolism which enhances the effect of the contraceptives. The decrease in microsomal cyt. P-450 level under imipramine treatment (17.5 nmoles/g wet liver against 22 nmoles/g in the controls; $P < 0.05$) supported this hypothesis and agreed with the known metabolic inhibition of numerous drugs by imipramine [21].

In summary, imipramine has been shown to modify the subcellular distribution of GGT in rat liver which resulted in the release of the enzyme from plasma membranes to cytosol and also in the increase of its activity in the plasma. Imipramine also enhanced the inducing effect of contraceptive steroids (MNm) on GGT activity. From these results we suggest that the potent alterations of liver plasma membranes caused by imipramine may be reflected in the increase of GGT activity in the plasma.

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