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# SHORT COMMUNICATIONS

## A study on the effect of chlorpromazine and desipramine on the activity of some adrenal enzymes

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Chlorpromazine and desipramine are very lipid-soluble compounds and have been shown to interfere with various oxidation and phosphorylation reactions in liver and brain mitochondria [1, 2]. Treatment with chlorpromazine increases liver tyrosine hydrogenase and glucose-6-phosphate dehydrogenase activity, as well as that of the mixed function oxidases involved in drug metabolism [3–5]. We have previously shown that chronic chlorpromazine or desipramine treatment decrease corticosterone production and that this decrease in steroidogenesis might be due to a direct action on the biosynthetic processes [6]. The biosynthesis of steroid hormones involves a series of stepwise hydroxylation processes which are known to require NADPH. In the rat adrenal cytosol, oxidation of isocitrate by NADP-linked isocitrate dehydrogenase is a major pathway for the production of NADPH, while the conversion of malate to pyruvate by cytosol malic enzyme also generates NADPH [7]. At the same time the citrate acid cycle enzymes and the cytochrome P-450 system were also known to be involved in steroid biosynthesis. In this investigation, we studied the *in vitro* and *in vivo* effect of chlorpromazine and desipramine on the activities of the adrenal NADP-isocitrate dehydrogenase (EC 1.1.1.42), malic enzyme NADP-malate dehydrogenase (EC 1.1.1.40), succinate dehydrogenase (EC 1.3.99.1) and the mitochondria cytochrome P-450.

Rats were treated with 10 mg/kg of chlorpromazine (Largactil, M & B) or desipramine (Pertofran, Ciba-Geigy) intraperitoneally once daily for two weeks. Control rats were injected with 0.2 ml saline intraperitoneally. At the end of this treatment period, the rats were killed and the adrenals were removed. Adrenal cytosol and mitochondrial fractions for enzymatic studies were obtained by differential centrifugation.

The NADP-isocitrate dehydrogenase and malic enzyme activity were assayed *in vitro*. A 0.5 ml aliquot of adrenal mitochondrial or cytosol fraction was mixed with 100 μl of isocitrate medium containing 2.3 μmoles of sodium isocitrate and 4 μmoles $MgCl_2$ dissolved in Tris–HCl buffer, or 100 μl of malate medium containing 5 μmoles of sodium malate and 5 μmoles $MgCl_2$ dissolved in Tris–HCl buffer pH 7.4 and pre-incubated in a water bath for 15 min at 37°. The reaction was started on addition of 20 μl of 0.5 μM NADP and the amount of NADPH formed was quantitated by measuring the fluorescence at excitation wavelength 340 nm and emission wavelength 455 nm (Aminco–Bowman spectrofluorometer). The enzyme activities were expressed as nmoles NADPH formed per mg protein per min.

To study the *in vitro* effect of chlorpromazine and desipramine on adrenal enzyme activity, chlorpromazine or desipramine was added to the assay mixture in small volumes of 5–20 μl to give the specified final concentrations. An enzyme assay was then performed as described.

The activity of adrenal mitochondrial succinate dehydrogenase was assayed according to the method of Kearney and Singer [8], and expressed as mmoles of DCIP reduced per mg protein per min using the extinction coefficient for DCIP as $19.1 \times 10^3\ M^{-1}$. The cytochrome P-450 content was determined by the method of Omura *et al.* [9].

*Results and discussion.* In the rat adrenal, the cytosol NADP-isocitrate dehydrogenase activity was much higher than that in the mitochondrial fraction. In chlorpromazine-treated but not desipramine-treated rats, the activity of NADP-isocitrate dehydrogenase in the cytosol fraction was decreased to 85 per cent of that of the corresponding control animals, whereas the decrease in mitochondrial isocitrate dehydrogenase activity was not statistically significant (Table 1). The malic enzyme activity in the rat adrenals was only detectable in the cytosol fraction. Chlorpromazine but not desipramine treatment decreased the adrenal cytosol malic enzyme activity significantly (Table 1). Since chlorpromazine treatment is known to decrease adrenal steroid production [6], one is tempted to suggest that chlorpromazine or its metabolites may have a direct inhibitory action on the NADPH generating system to result in a decrease in hydroxylation and subsequently steroidogenesis. On the other hand, a

Table 1. Effect of chlorpromazine and desipramine treatment on the adrenal isocitrate dehydrogenase, malic enzyme and succinate dehydrogenase activities

| Treatment | Isocitrate dehydrogenase (NADPH formed: nmoles/mg Pr/min) | | Malic enzyme (NADPH formed: nmoles/mg Pr/min) | Succinate dehydrogenase (DCIP reduced: mmoles/mg Pr/min) |
|---|---|---|---|---|
| | Cytoplasmic | Mitochondrial | | |
| Control | 56.67 ± 2.32 (10) | 38.18 ± 3.77 (12) | 33.56 ± 3.55 (8) | 0.482 ± 0.015 (12) |
| Chlorpromazine | 47.12 ± 1.76* (9) | 28.22 ± 7.16 (N.S.) (12) | 24.14 ± 2.36* (8) | 0.448 ± 0.016 (12) |
| Control | 60.00 ± 1.69 (6) | 30.9 ± 3.88 (6) | 31.10 ± 1.068 (8) | 0.370 ± 0.026 (6) |
| Desipramine | 61.50 ± 0.87 (6) | 33.6 ± 5.87 (6) | 31.83 ± 1.87 (8) | 0.389 ± 0.19 (6) |

Numbers in parentheses are the numbers of animals in each group.
* $P < 0.05$. N.S.—not significant.

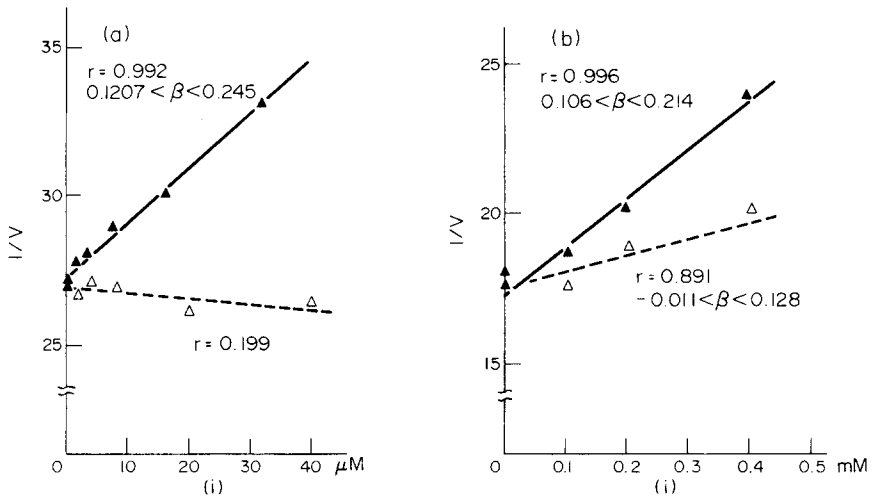


Fig. 1. The *in vitro* effect of chlorpromazine (−▲−▲−) and desipramine (−△−△−) on adrenal NADP-isocitrate dehydrogenase activity (a) and malic enzyme activity (b). (*i*) = final concentration of chlorpromazine or desipramine in the incubation medium. *v* = NADPH formed, in nmoles/mg Pr/min. *r* is the correlation coefficient; *β* is the 95% confidence interval for the slope of the regression line.

decrease in NADP-isocitrate dehydrogenase activity has been found in hypophysectomised rats secondary to the reduction of cholesterol demolase activity [10]. Thus the observed decrease in NADP-isocitrate dehydrogenase and malic enzyme activity might be secondary to the inhibition of steroid production by chlorpromazine at other sites. It is interesting to note that desipramine treatment has no effect on these enzymes although the corticosterone production is also inhibited [11].

In *in vitro* experiments, mitochondrial NADP-linked isocitrate dehydrogenase activity was inhibited in a dose-dependent manner by chlorpromazine concentrations ranging between 1 and 32 μM (Fig. 1(a)). This is a relatively low concentration compared with the concentration of chlorpromazine reported to inhibit liver aspartate transaminase which is also a soluble mitochondrial enzyme [12]. In spite of a significant reduction in malic enzyme activity in chlorpromazine-treated rat adrenals, a chlorpromazine concentration of 0.1 mM and above was required to inhibit malic enzyme *in vitro* (Fig. 1(b)). Within the dose ranges cited for chlorpromazine, desipramine had no significant inhibitory effect on NADP-isocitrate dehydrogenase and malic enzyme activity *in vitro* (Fig. 1(a) and (b)), and chlorpromazine appears to be a more potent inhibitor of the adrenal soluble enzymes studied. Such a potency difference was not observed between impramine and chlorpromazine on the membrane bound enzyme NAD–ATPase and succinate dehydrogenase of the liver [12]. Our studies showed that the adrenal succinate dehydrogenase activity was inhibited at desipramine concentrations of 0.1–0.4 mM (Fig. 2), a concentration range comparable to that of imipramine and chlorpromazine reported to inhibit this enzyme in liver and brain mitochondria. Unfortunately in our system, chlorpromazine was precipitated at concentrations above 0.1 mM and its effect on adrenal succinate dehydrogenase was not estimated above this concentration. At chlorpromazine concentrations below 0.1 mM, the adrenal succinate dehydrogenase activity was not inhibited. Neither chlorpromazine nor desipramine treatment had any effect on adrenal succinate dehydrogenase activity. Thus it appears that chlorpromazine and desipramine have similar effects on the membrane bound enzyme succinate dehydrogenase but differ in their action on the soluble enzyme both *in vivo* and *in vitro*.

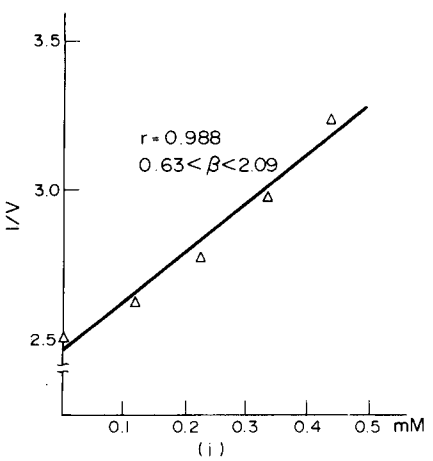


Fig. 2. The relationship between adrenal succinate dehydrogenase activity and desipramine *in vitro*. (*i*) = final concentration of desipramine added to incubation medium. *v* = DICP reduced in mmoles/mg Pr/min. *r* is the correlation coefficient; *β* is the 95% confidence interval for the slope of the regression line.

The rate limiting process in steroidogenesis is the conversion of cholesterol to pregnanolone by cholesterol demolase in the mitochondria with cytochrome P-450 as the terminal electron donor. ACTH treatment and drugs such as chloramphenicol change the amount of cytochrome P-450 in the mitochondria [13]. The liver and adrenal mitochondrial cytochrome P-450 content was measured. In normal rat adrenal the mitochondria cytochrome P-450 content was found to be 0.597 ± 0.074 nmoles/mg Pr, whereas in the chlorpromazine-treated rats, the cytochrome P-450 content was found to be 0.692 ± 0.058 nmoles/mg Pr. The liver microsomal P-450 content was found to be the same in treated and control rats, being 1.14 ± 0.05 nmoles/mg Pr and 1.14 ± 0.06 nmoles/mg Pr respectively. Thus chlorpromazine treatment did not induce a decrease in mitochondria cytochrome P-450 content.

Preliminary experiments showed that chlorpromazine binds to mitochondria P-450 to form a type II spectrum,

whereas both cholesterol and deoxycorticosterone form a type I spectrum. It is unlikely that chlorpromazine inhibits steroidogenesis by inhibiting P-450 availability.

In summary, chlorpromazine treatment was found to decrease adrenal cytosol isocitrate dehydrogenase and malic enzyme activity. Chlorpromazine also had a direct action *in vitro* to inhibit the activity of these enzymes. Desipramine treatment had no effect on the soluble enzymes studied and the concentration needed to inhibit succinate dehydrogenase in the adrenal was similar to that reported for the liver and brain mitochondria enzyme. Chlorpromazine treatment did not decrease P-450 content. It is not known whether the influence of chlorpromazine and desipramine on adrenal enzymes is related to their inhibitory effects on steroid production.

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# Glutathione-*S*-transferase in human fetal liver*

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Part of the carcinogenic and mutagenic properties of polycyclic aromatic hydrocarbons have been ascribed to their epoxide metabolites [1–3]. Arene oxides have also been implicated as causative agents in tissue necrosis [4]. Little is known, however about the effects of such epoxides on the human fetus.

In view of the fact that the human fetal liver contains the monooxygenase enzyme system [5] and catalyzes the formation of epoxides [6–8] we have investigated the further metabolism of epoxides in preparations of human fetal liver. Apart from being rearranged in water to form phenols, arene oxides may be enzymatically converted to dihydrodiols by a microsomal epoxide hydratase (EC 3.3.2.3) [9] or to glutathione conjugates by glutathione-*S*-transferase (EC 2.5.1.18) [10] which resides in the soluble fraction of the hepatocytes [11, 12].

Multiple basic [13] and recently a neutral [14, 15] form of the glutathione-*S*-transferase were characterized in the human liver. The enzyme has also been described in erythrocytes [16] and placenta [17]. Although the ontogenic post-natal development of hepatic glutathione-*S*-transferase has been studied in rats [18, 19] there is virtually no information on the development of this enzyme in man apart from one preliminary report on the presence of glutathione-*S*-naphthalene-1,2-oxide transferase in a single human fetal liver specimen [20].

This report describes the results from an investigation of glutathione-*S*-transferase in several different human fetal liver preparations.

Liver specimens were obtained from human fetuses at legal abortion on socio-medical indications by Cesarean section or prostaglandin or ethacridine (Rivanol®) induction. The gestational ages of the fetuses varied between 10 and 27 weeks. One third of the mothers were regular smokers and one (no. 12) received $\beta_2$-receptor stimulating agents because of asthma.

Specimens of the livers were excised within 45 min of the death of the fetus and frozen at minus 80° if not used at once. After homogenization of the liver piece in 0.25 M sucrose (1:3, w/v) and centrifugation at 9000 *g* for 10 min the 9000 *g* supernatant was centrifuged for 60 min at 105,000 *g* and the supernatant used for the incubations. All steps were carried out at a temperature of 0–4°.

The enzyme assay was essentially the same as described previously [21] with minor modifications. In brief, incubation mixtures of 100 μl contained 0.1 M sodium pyrophosphate buffer, pH 8.5, cytosolic protein at final concentrations between 2 and 6 mg per ml, and 5 μl of acetonitrile containing various amounts of styrene-7, 8-oxide (SO†, to give a final concentration of 6 mM or, when varied, between 0.5 and 12 mM) and about 100,000 cpm of [7-$^3$H]styrene oxide (Radiochemical Centre, Amersham, U.K., sp. act. 99.6 mCi per mmole). The reaction was started by adding 20 μl distilled water containing various

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† Abbreviations used: SO = styrene-7,8-oxide; $K_m$ = Michaelis–Menten constant; $V$ = velocity of the enzyme reaction.