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A STUDY ON THE EFFECTS OF CHLORPROMAZINE AND DESIPRAMINE ON RAT ADRENAL STEROIDOGENESIS AND CHOLESTEROL ESTERASE ACTIVITY

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Drugs used in the treatment of psychiatric illness were shown to have an effect on the hypothalamic–pituitary–adrenal axis [1]. Thus chlorpromazine treatment was associated with a decrease in 17-OH steroid excretion, [2, 3] while a decrease in plasma cortisol was reported in patients treated with tricyclic antidepressants [4]. Previous works in this laboratory, demonstrated a decrease in corticosterone and an accumulation of cholesterol esters in the adrenal glands of rats chronically treated with chlorpromazine and desipramine [5]. It is not known whether the decrease in adrenal corticosterone was due to a reduced rate of conversion of cholesteryl ester to free cholesterol, the substrate for corticosterone synthesis. This study aims at further delineating the effect of these drugs on cholesterol esterase activity and corticosterone production in the adrenal gland.

Male Sprague–Dawley rats weighing 180–250 g were used. Rats were treated with 10 mg/kg of chlorpromazine (Largactil, M & B) or desipramine (Pertofran, Ciba-Geigy) intraperitoneally, twice daily for 14 days. At the end of the treatment period, rats were decapitated and adrenals removed, trimmed of adherent fat and used for the assay of cholesterol esterase activity or steroidogenesis activity.

In steroidogenesis studies, each adrenal gland was sliced in six slices and preincubated in 1 ml Krebs–Ringer bicarbonate medium pH 7.4 for 30 min [6, 7]. After the preincubation period, the fluid was removed and fresh Krebs–Ringer bicarbonate medium was added. Fifty μl of cholesterol (1 mg/ml) in 50% ethanol in a final concentration of 1.22 mM was added and incubation was performed at 37° in an atmosphere of 95% O_2 and 5% CO_2 for 2 hr. In one series of experiment, 0.5 I.U. ACTH (Acthar Gel, Armour) was added to the incubation medium. In *in vitro* studies of drug effect on steroid synthesis, normal untreated rat adrenals were used for the incubation and a fixed volume (5 μl) of chlorpromazine or desipramine in various concentrations was added to the incubation vial containing one adrenal while 5 μl saline was added to the vial containing the contralateral adrenal which was used as control in a paired study design. At the end of 2 hr, the adrenal slices were homogenised and the combined adrenal homogenate and incubation fluid from each vial were extracted with methylene chloride. Aliquots of methylene chloride extract were taken for fluorometric determination of corticosterone by the method of Frankel, Graber & Nalbandov 1967 [8].

The cholesterol esterase (EC 3.1.1.13) activity was assayed by a modify method of Goodman 1969 [9]. Each adrenal was homogenised in Tris–HCl buffer pH 7.4 (10 mg/2 ml w/v) and centrifuged at 9000 *g* for 15 min. The supernatant, containing the cytoplasmic fraction was used in the cholesterol esterase assay. 0.5 ml of cytoplasmic fraction (protein concentration 1.0 mg/ml) was added to 0.48 ml of incubation medium containing 2.5 μmoles of mercaptoethanol, 7.5 μmoles $MgCl_2$ and 3.7 μmoles KCl dissolved in 0.3 M Tris–HCl buffer (pH 7.4). Incubation was started on addition of 20 μl of 4-^{14}C-cholesteryl oleate (0.743 mM, 5×10^7 ct/min/ml) in acetone. After 1 hr incubation, at 37°, the reaction was stopped on addition of 10 ml chloroform : methanol (2 : 1 v/v) mixture. The unchanged cholesteryl oleate and the free cholesterol formed during incubation were extracted into the organic layer and separated by column chromatography using an aluminium oxide column. The residual cholesteryl oleate was eluted with petroleum ether : benzene (1 : 1 v/v) mixture and the free cholesterol formed was eluted with acetone : diethyl ether (1 : 1 v/v) mixture. The radioactivity of the eluates was determined by liquid scintillation counting (ICN, Tracerlab). In *in vitro* studies of drug effect on the enzyme activity, pooled adrenal cytoplasmic fractions were used in the assay and a fixed volume of saline or drug was added to the incubation medium. The colesteryl esterase activity was expressed as the amount of free cholesterol formed nmol/mg Pr/hr.

RESULTS AND DISCUSSION

In these studies, the rate of corticosterone synthesis by chlorpromazine and desipramine treated rat adrenals was found to be not significantly different from that of control rats and the adrenal response to ACTH added *in vitro* was not diminished in adrenals of drug treated rats (Table 1). These results were unexpected as chlorpromazine treatment not only was associated with a decrease in plasma and adrenal corticosterone concentrations, it was also shown to suppress the adrenal response to exogenous ACTH *in vivo*, indicating that it had a direct action on the adrenals [5, 10]. A decrease in adrenal isocitrate dehydrogenase and malic enzyme activity was demonstrated in chlorpromazine

Table 1. Effect of chronic chlorpromazine and desipramine treatment on steroidogenesis, and cholesterol esterase activity

Treatment	Corticosteroid formed (μg/10 mg adr./hr)		Cholesterol esterase activity†
	Adrenals	Adrenals + ACTH*	
n	6	6	12
Control	1.49 ± 0.20	5.75 ± 0.62	9.09 ± 0.82
Chlorpromazine treated	2.04 ± 0.25[N.S.]	5.58 ± 0.42	8.23 ± 0.67[N.S.]
n	6	6	6
Control	2.27 ± 0.36	4.73 ± 0.29	11.46 ± 1.75
Desipramine treated	1.58 ± 0.17[N.S.]	4.13 ± 0.48	14.78 ± 1.65[N.S.]

* ACTH 0.5 I.U. was added *in vitro* to the incubation medium.

† Cholesterol esterase activity was measured by the amount of free cholesterol formed nmol/mg Pr/hr in the assay system.

treated rats [11]. The failure of this study to demonstrate an inhibition in the conversion of cholesterol to corticosterone by drug treated rat adrenals might be explained either by the fact that the drug accumulated in the tissue was diluted in the incubation medium to a concentration that is no longer inhibitory or the cholesterol substrate and oxygen supplied served to reverse the chlorpromazine or desipramine induced inhibition. When chlorpromazine or desipramine was added *in vitro* to the incubation medium, corticosterone synthesis was not inhibited even at chlorpromazine concentration of 0.21 mM. With increase in chlorpromazine concentration to 0.84 mM a stimulation of corticosterone synthesis was observed (Table 2). Desipramine when added *in vitro* caused a significant increase in corticosterone production at 0.132 mM (Table 2). In either case drug concentrations below 0.1 mM induced no significant changes in corticosterone production in the incubation system used. While it is difficult to relate the drug concentrations used *in vitro* system to *in vivo* situation, the *in vitro* results would suggest that the lack of inhibition in the conversion of cholesterol to corticosterone by adrenal slices might not be due to the low concentration of drug present. The stimulation of corticosterone production at high drug concentrations was more difficult to understand. In the presence of ACTH, chlorpromazine at 0.21 mM suppressed the ACTH induced increase in corticosterone production to the order of 50% (Table 2). It is not known whether chlorpromazine inhibits the activation of enzymes by ACTH or the observed effect is secondary to its inhibitory action on NADPH generating enzymes [11] which might become rate limiting when the synthetic rate is high.

In the adrenal, the free cholesterol for steroidogenesis is mainly provided by hydrolysis of the stored cholesterol esters by cholesterol esterase [12]. Administration of ACTH resulted in a significant enhancement of cholesterol esterase activity and a depletion of cholesterol esters within the adrenal gland [13]. Chronic chlorpromazine and desipramine treatment markedly increase the cholesterol ester level in the adrenal cytoplasmic fraction [5]. However, using cholesterol oleate as substrate, the cholesterol esterase activity in drug treated rat adrenals was found to be similar to that of control rats (Table 1). It thus appeared that the decrease in plasma and adrenal corticosterone levels is not due to a decrease

Table 2. Effect of chlorpromazine and desipramine on steroidogenesis *in vitro*

	Corticosterone formed (μg/10 mg adr./hr)		
n = 6	0.21 mM	0.42 mM	0.84 mM
Control	1.49	0.711	0.848
Chlorpromazine	1.39	0.642	1.121
Mean difference ±S.D.	−0.028 ± 0.0424	0.067* ± 0.044	−0.273† ± 0.036
n = 6			
Control + ACTH	4.918	5.717	4.720
Chlorpromazine	2.796	2.352	2.033
Mean difference ±S.D.	2.122* ± 0.437	3.352* ± 0.612	2.686* ± 0.685
n = 6	0.066 mM	0.132 mM	
Control	1.577	1.417	
Desipramine	1.899	1.850	
Mean difference ±S.D.	−0.312 ± 0.235	−0.433* ± 0.103	

* $P < 0.01$; † $P < 0.001$ as compared with contralateral control adrenal in a paired Student's *t*-test.

in turn over of cholesterol ester to free cholesterol, the substrate for corticosterone synthesis. The accumulation of cholesterol ester in drug treated rat adrenals might be an effect of these compounds on cholesterol ester uptake or synthesis and is independent of the corresponding changes in plasma and adrenal corticosterone levels.

When chlorpromazine was added *in vitro*, a biphasic effect on cholesterol esterase activity was observed (Fig. 1). At chlorpromazine concentrations below 0.2 mM, a sharp fall in cholesterol esterase activity was obtained and a nadir was reached around 0.2–0.4 mM, while with further increase in chlorpromazine dose, the inhibitory effect decreased and the percentage of inhibition at 1.2 mM was similar to that of 0.08 mM chlorpromazine. With desipramine, a biphasic inhibition–activation curve was obtained. At desipramine concentrations below 0.1 mM an inhibition was observed and at 0.1 mM the inhibition was over 30%. At desipramine concentrations above 0.3 mM, desipramine appeared to stimulate the cholesterol esterase activity (Fig. 1). Thus chlorpromazine and desipramine can inhibit cholesterol esterase activity. However, the dose at which it will do so is fairly high in comparison with the organophosphate, dichlovos [14]. In view of the high concentrations required, the observed inhibitory action might not be those involved in the inhibition of steroid production. The inhibition–activation phenomenon seems to be incompatible with any mechanism involving specific chemical intervention on an enzyme system. Since both chlorpromazine and desipramine are very lipid soluble substances, it is possible that the activation observed at high doses of drug might be a physical phenomenon related to the increase solubilization of substrate cholesteryl ester.

In summary, this study demonstrates the inherent difficulty in relating *in vivo* observations to drug action in the *in vitro* system. In the presence of cholesterol and oxygen, the rate of conversion of cholesterol to corticosterone by adrenal slices of chlorpromazine or desipramine treated rats was similar to those of control rat adrenals. Addition of chlorpromazine *in vitro* did not inhibit corticosterone production in normal rat adrenals but will inhibit ACTH stimulated steroid production. Desipramine added *in vitro* stimulated steroid production. Chlorpromazine and desipramine were shown to inhibit cholesterol esterase activity *in vitro* but the cholesterol esterase activity was not decreased in drug treated rat adrenals. Thus the decrease in plasma and adrenal corticosterone levels in chlorpromazine and desipramine treated rats might be unrelated to the accumulation of cholesterol ester or to the inhibition of cholesterol esterase activity by these drugs.

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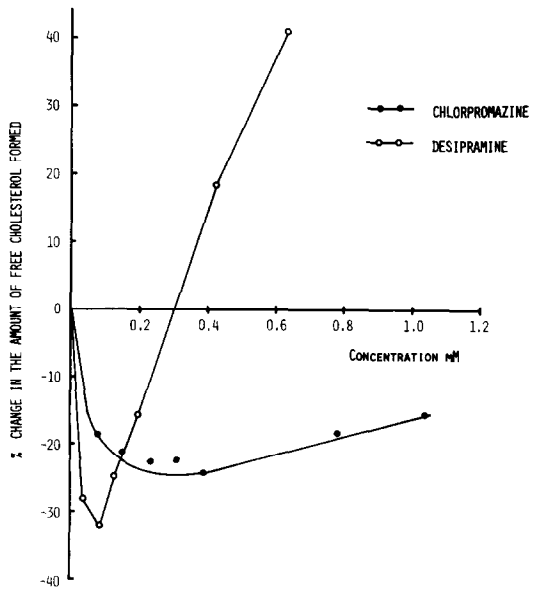

Fig. 1. Effect of chlorpromazine and desipramine on cholesterol esterase activity *in vitro*. Each point was the mean of two duplicate determinations.

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