CHAPTER - V

EFFECTS OF PREGNENOLONE AND CORTISOL ON HEPATIC MIXED FUNCTION OXIDASE SYSTEM AND SERUM AND TISSUE LEVELS OF ASCORBIC ACID

Introduction:

The occurrence of ascorbic acid in the living cells is ubiquitous although its levels vary considerably from tissue to tissue. Ascorbic acid in the adrenal glands is found to be greater than in any other tissue. The effect of stress or ACTH administration decreased the level of ascorbic acid in adrenal glands (1-3). Slusher and Roberts (4) showed that ascorbic acid level was closely related to the cortical metabolism in adrenal glands. Bronson and Eleftheriou (5), Jones et al. (6) and several others (7-12) claimed that ascorbic acid played an important part in oxidation-reduction reactions and thus influenced adrenal steroidogenesis. Bjorkhem and Kallner (13) reported that adrenal ascorbate might be playing an important role in the conversion of cholesterol to pregnenolone, a rate limiting step in the steroid bio-synthesis (14,15).

Marion et al. (16) observed that ascorbic acid produced an increase of cyclic AMP by inhibiting 3',5' cyclic AMP phosphodiesterase. Cyclic AMP has been demonstrated to be mediator of the steroidogenic action of ACTH (1,17-19). Again it has been reported that ascorbic acid inhibits adrenal steroidogenesis (20,21). Laskava and Lyesnyk (22) observed that massive dose of ascorbic acid in rats altered the levels of circulating steroid hormones in blood.
These reports suggest the possibility of blood steroid hormones level being affected during ascorbic acid administration. It appears that induced effect of ascorbic acid on hepatic mixed function oxidase system might be due to the altered level of blood steroid hormones. Data pertaining to these aspects are not available. Hence the present studies were designed to investigate the effects of steroid hormones on the hepatic mixed function oxidase system and serum and tissue levels of ascorbic acid.

Materials and Methods:

Hindustan Antibiotic strain, male rats (obtained from Hindustan Antibiotics Ltd., Pune), weighing 120-130 g, were used in the present experiments. They were housed in an air-conditioned room and fed a standard laboratory diet (obtained from Hindustan Lever, Bombay) and water ad libitum for 10-15 days prior to the initiation of the experiments. The male rats were classified into the following groups:

1. Control group of animals:

The control group of animals received only olive oil twice daily at 6.00 a.m. and 6.00 p.m. for 5 successive days.

2. Ascorbic acid treated animals:

In this group, animals were injected with ascorbic acid (87.5 mg/kg body wt.) twice daily as above mentioned timings for 5 successive days.
3. Pregnenolone treated group of animals:

The rats were injected i.p. with pregnenolone in olive oil twice daily at 6.00 a.m. and 6.00 p.m. for 5 successive days with the doses of 1 mg and 0.1 mg per animal.

4. Cortisol treated group:

The animals from this group were similarly injected with cortisol in olive oil and the dose levels were 1 mg and 0.1 mg cortisol per animal.

The animals were sacrificed 12 hours after the last treatment by cervical dislocation. Blood was collected from each sample as soon as possible. The livers were perfused with 0.9% ice-cold saline, isolated and weighed. Kidney and adrenals were isolated and were chilled at -4°C.

Ascorbic acid levels in the livers, kidney, adrenals and serum were determined as described under 'Materials and Methods' of Chapter I.

A part of the livers was homogenized (1:4 w/v) in ice-cold 50mM tris-HCl buffer, pH 7.4 containing 1.15% KCl. The microsomes were isolated as described under 'Materials and Methods' of Chapter I.

The microsomal pellets were resuspended in 0.25M sucrose. Microsomal protein was estimated according to the biuret method (23) using crystalline bovine serum albumin as the standard.
The hepatic microsomal aminopyrine N-demethylase and acetanilide hydroxylase activities were determined essentially as described in Chapter-I, using NADPH as electron donor.

Formaldehyde produced during the N-demethylation reactions was measured according to the method of Nash (24), and p-OH acetanilide produced by hydroxylation was determined by the method of Weisburger and Goodall (25) as described in Chapter-I.

The levels of microsomal NADPH cytochrome c reductase, cytochrome b$_{5}$ and cytochrome P-450 were determined as described under 'Materials and Methods' in Chapter-I.

Statistical analyses were carried out according to the modified students 't' test (26).

RESULTS

The effect of ascorbic acid on the activities of drug metabolizing enzymes and levels of microsomal electron transport components have been included under Chapter-I.

Effect of ascorbic acid, pregnenolone and cortisol on liver, adrenals and serum ascorbic acid content:

Ascorbic acid treatment did not cause any significant change in the liver ascorbic acid. However, adrenals and serum ascorbic acid levels were significantly increased. The percentage of increase in adrenals and serum ascorbic acid
Table 1

Effect of ascorbic acid, pregnenolone and cortisol treatment on ascorbic acid levels in various tissues

<table>
<thead>
<tr>
<th>Group</th>
<th>Ascorbic acid levels</th>
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<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>µg AsA/g tissue</td>
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<tr>
<td>1. Control</td>
<td>230.8 ± 6.2</td>
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<tr>
<td>2. Ascorbic acid treated 87.5 mg AsA/kg b. wt.</td>
<td>235.2 ± 8.8 NS</td>
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<tr>
<td>3. Pregnenolone treated (1 mg/animal)</td>
<td>240.4 ± 4.2 NS</td>
</tr>
<tr>
<td>4. Pregnenolone treated (0.1 mg/animal)</td>
<td>241.4 ± 3.2 NS</td>
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<tr>
<td>5. Cortisol treated (1 mg/animal)</td>
<td>239.2 ± 5.2 NS</td>
</tr>
<tr>
<td>6. Cortisol treated (0.1 mg/animal)</td>
<td>240.5 ± 4.2 NS</td>
</tr>
</tbody>
</table>

Values are mean ± SE, 6 animals in each group.
All values are statistically significant at P ≤ 0.05.
NS = not significant.
content was 37 and 38 respectively. Administration of both cortisol and pregnenolone resulted in a marginal change in the liver and adrenals ascorbic acid content. The highest magnitude of increase in liver ascorbic acid content was by 3% registered with 1 mg per animal pregnenolone injection. The overall increase in liver ascorbic acid was of the order of 6% with 0.1 mg cortisol administration. Pregnenolone at a dose of 1 mg per ml caused a marginal decrease in the adrenal ascorbic acid content. However, the next dose of 0.1 mg resulted in an increase of 10% in adrenal ascorbic acid content. Serum ascorbic acid content was unaltered during pregnenolone or cortisol treatment. However, with the last dose of 0.1 mg cortisol, a 9% increase was noted. Kidney ascorbic acid level remained unaltered during cortisol and pregnenolone treatment.

Effect of pregnenolone and cortisol on microsomal protein content:

Pregnenolone treatment resulted in a decrease in microsomal protein content. At a low dose of 0.1 mg a 16% decrease was noted. The next dose of 1.0 mg pregnenolone per animal caused a further decrease in microsomal protein content. However, cortisol at low dose of 0.1 mg caused an increase of the microsomal protein content by 24%. Cortisol dose of 1 mg caused a further increase of microsomal protein content by 60% as compared to control group of animals.
MICROSOMAL PROTEIN, DRUG METABOLIZING ENZYMES
ACTIVITY DURING ASA, PREGNENOLONE & CORTISOL
TREATMENT.
1. CONTROL. 2. ASA TREATED. 3. PREGNENOLONE (1 mg)
4. PREGNENOLONE (0.1 mg). 5. CORTISOL (1 mg). 6. CORTISOL (0.1 mg)
LEVELS OF ELECTRON TRANSPORT COMPONENTS DURING ASCORBIC ACID PREGNENOLONE AND CORTISOL TREATMENT.

Cyt. c reductase  Cyt. b$_5$  Cyt. P$_{450}$

% Control

1. control.  2. AsA treated.  3. Pregnenolone (1 mg)  4. Pregnenolone (0.1 mg).  5. Cortisol (1 mg)  6. Cortisol (0.1 mg)
Effect of pregnenolone and cortisol on the activities of drug metabolizing enzymes:

A significant decrease in aminopyrine N-demethylase and acetonilide hydroxylase activities were observed during 1 mg pregnenolone treatment in male rats. The percentage of decrease in aminopyrine N-demethylase activity was 20 and acetonilide hydroxylase activity was 30. However, 0.1 mg pregnenolone treatment resulted an insignificant change in the activities of aminopyrine N-demethylase and acetonilide hydroxylase. The activities of aminopyrine N-demethylase and acetonilide hydroxylase were increased during cortisol treatment. In the animals treated with 1 mg and 0.1 mg cortisol, the percentage of increase in aminopyrine N-demetahyldes activities was 60 and 20 respectively. Acetonilide hydroxylase activity was increased by 75% and 30% in the rats treated with 1 mg and 0.1 mg cortisol respectively.

Effect of pregnenolone and cortisol treatment on electron transport components in male rats:

The cytochrome c reductase activity was decreased in pregnenolone treated male rats. The percentage of decrease was 24 and 35 during 0.1 mg and 1 mg pregnenolone treatment respectively. The cytochrome c reductase activity was increased by 35% in 1 mg cortisol treated rats and 20% in 0.1 mg cortisol treated rats. During 1 mg pregnenolone administration, the level of cytochrome b$_5$ was
decreased by 12% in male rats. However, there was a marginal increase of 3% in 0.1 mg pregnenolone treated rats. Cortisol treatment resulted in an increase in cytochrome b\textsubscript{5} level. The percentage of increase was 30 and 8 during 1 mg and 0.1 mg cortisol treatment respectively. The cytochrome P-450 content was decreased by 25% during 1 mg pregnenolone treatment and it was increased by 20% during 1 mg cortisol treatment. However, 0.1 mg pregnenolone or cortisol treatment resulted in an insignificant change in cytochrome P-450 level in male rats. The cytochrome P-450 content was decreased by 30% during 1 mg pregnenolone treatment and increased by 28% during 1 mg cortisol treatment.

**DISCUSSION**

The selective accumulation of ascorbic acid by adrenal glands during ascorbic acid administration was observed. The function of ascorbic acid in the adrenal glands is not yet fully known. However, it has been recently reported that the plasma levels of adrenocorticoid were elevated with massive dose of ascorbic acid administered with diet (28). Laskava and Lyesnyk (22) reported that ascorbic acid administration resulted alteration in the levels of steroid hormones in blood. Thus it suggests that ascorbic acid may interact with adrenal glands to effect changes in the adrenal hormone synthesis and/or secretion.

The inhibition of hepatic drug metabolizing enzymes due to pregnenolone administration and induction due to cortisol
treatment observed could be because of their binding to the F3 histones of the liver at the transcription level to alter de novo synthesis of enzyme protein (29). Similar observations were also reported by Lester and Fred (30). Steroid hormones also regulate the pattern of hepatic enzymes syntheses by altering the smooth membranes of the liver endoplasmic reticulum (31).

The observed alteration in the activities of drug metabolizing enzymes without significant change in serum, liver and adrenal ascorbic acid levels could indicate that ascorbic acid has no role in the induction of mixed function oxidase system during these steroid hormones administration.

Castro et al. (32) observed that adrenalectomy decreased the levels of cytochrome c reductase and cytochrome P-450 which were restored by corticosterone treatment, in adrenalectomized rats and by both corticosterone and ACTH in hypophysectomized animals and further suggested that the pituitary-adrenal system exerts a regulatory function on the hepatic drug metabolizing enzymes. This observation was confirmed by several other investigators (33-39). The inhibition of drug metabolizing enzymes observed during pregnenolone administration and induction by cortisol could suggest that increase in the ratio of pregnenolone to cortisol in blood could induce hepatic mixed function oxidase system. The alteration in the levels of steroid hormones in blood during contrary? cortisol to pregnenolone, I suppose.
Ascorbic acid administration in rats has been recently reported (22,28). This suggests that the induction of hepatic drug metabolizing enzymes activities observed during ascorbic acid administration could be mediated through adrenal hormones by altering the balance between the levels of pregnenolone and cortisol. The role of other adrenal steroid hormones which are possibly altered, on hepatic mixed function oxidase system during ascorbic acid administration cannot be ruled out. However, a detailed analysis of steroid hormones during ascorbic acid administration will be needed to evaluate the possible role of adrenal steroid hormones in the induction of hepatic mixed function oxidase system during ascorbic acid administration.
SUMMARY

The effects of ascorbic acid and different doses of pregnenolone and cortisol on serum and tissue levels of ascorbic acid, activities of hepatic drug metabolizing enzymes and the levels of microsomal electron transport components were examined in male rats.

The present studies indicate that

(1) Ascorbic acid treatment resulted in an increase in adrenals and serum ascorbic acid content while liver ascorbic acid content was not altered;

(2) Pregnenolone or cortisol administration had no effect on tissue levels of ascorbic acid;

(3) Pregnenolone at a dose of 1 mg showed a decrease in the activities of aminopyrine N-demethylase and acetonilide hydroxylase and levels of cytochrome P-450 and cytochrome c reductase whereas pregnenolone at a dose of 0.1 mg had no significant effect on these parameters of drug metabolism; and

(4) Cortisol treatment resulted in an increase in the activities of aminopyrine N-demethylase and acetonilide hydroxylase with parallel increase in cytochrome P-450 content.
REFERENCES


GENERAL CONCLUSION

All drugs have side effects in addition to their therapeutic efficiency. The drug therapeutic efficiency and the side effects are determined by the capacity of the individual to metabolize and excrete them. In recent years, millions consume large quantity of ascorbic acid to avoid side effects of drugs and toxicity of chemicals. However, the effect of ascorbic acid administration on hepatic drug metabolizing enzyme activities has not yet been fully investigated. The present study was planned to investigate the effect of ascorbic acid on the hepatic mixed function oxidase system.

Our present studies show that ascorbic acid administration resulted in an induction of hepatic mixed function oxidase system in rats, mice and guinea pigs. Liver ascorbic acid level was not increased during ascorbic acid administration. This observation led us to suggest that ascorbic acid induced hepatic mixed function oxidase system through mediator(s). Ascorbic acid pretreatment depressed the induction effect of phenobarbital and DDT in mice. Ascorbic acid after phenobarbital or DDT administration resulted in a further increase in the activity of microsomal enzymes. Ascorbic acid administration with phenobarbital or DDT caused maximum induction. The pattern of induction during ascorbic acid with phenobarbital or 3 methyl-cholanthrene administration was different.
These inducers stimulate ascorbic acid synthesis in liver, in addition to their effect on mixed function oxidase system. Additional administration of ascorbic acid resulted in a further increase in the activities of drug metabolizing enzymes. This result suggests that the apparent induction of drug metabolizing enzymes activities due to inducers could at least be partly due to their effect of stimulation of ascorbic acid synthesis in liver and its effect on microsomal enzymes.

During the early period of starvation, there was a parallel increase in the activities of drug metabolizing enzymes and serum ascorbic acid level.

Pregnenolone is a potent inhibitor and cortisol is an inducer of hepatic mixed function oxidase system. Since these steroid hormones do not alter ascorbic acid levels in liver and serum, ascorbic acid could not participate during these steroids administration for the alteration of hepatic mixed function oxidase system. Ascorbic acid administration increased adrenal ascorbic acid content. This suggests that adrenal steroid hormones synthesis and/or release was altered during ascorbic acid administration.

This observation has led us to postulate that changes in normal pregnenolone and cortisol balance could influence the metabolism of drugs and the pattern of liver response. This raised the possibility that ascorbic acid administration caused an induction of hepatic mixed function oxidase system.
by changing normal balance between pregnenolone and cortisol.

Marshall and McLean* proposed a model in which hydroxylating enzyme synthesis is controlled by an endogenous inducer. Our study supports the suggestion that the induction of hepatic mixed function oxidase system is at least partly mediated through an endogenous inducer and that this inducer is ascorbic acid.

Treatment of animals with wide variety of chemicals increases ascorbic acid synthesis in rats. Thus a new study state has been attained to get a maximum induction of mixed function oxidase system and to excrete foreign compounds. Ascorbic acid could induce hepatic mixed function oxidase by altering the levels of steroid hormones in blood. Then the exogenous inducer is cleared from the body and the stimulation of ascorbic acid synthesis is reduced. Ascorbic acid level is reduced to normal value. Since steroid hormones are substrate of the enzyme, the concentration of steroid hormones should decrease in the face of the increased enzyme activity and decreased competition from the exogenous inducers. This decrease in the concentration of steroid hormones to normal level should precede the return of enzymes activities to control values.