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Onset of Enzyme Induction with Pregnenolone-16α-carbonitrile in Male and Female Rats

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Abstract □ Male and female rats treated with a single 20-mg/kg ip dose of pregnenolone-16α-carbonitrile (PCN) produced parallel increases in hepatic cytochrome P-450 and aniline hydroxylase activity. However, the onset of increase in aniline hydroxylase activity and cytochrome P-450 content was slower in male than female animals. The maximal levels achieved in male rats were 89% of the values observed in the female animals. Furthermore, the hepatic aniline hydroxylase activity and P-450 content declined more rapidly in male than in female rats.

Keyphrases □ Pregnenolone-16α-carbonitrile—effect on hepatic cytochrome P-450 and aniline hydroxylase activity, male and female rats compared □ Cytochrome P-450 activity—effect of pregnenolone-16α-carbonitrile, male and female rats compared □ Aniline hydroxylase activity—effect of pregnenolone-16α-carbonitrile, male and female rats compared

3β-Hydroxypregn-5-ene-20-one-16α-carbonitrile, commonly known as pregnenolone-16α-carbonitrile or PCN (I), is a catatoxic steroid with no known hormonal activity. However, it is rapidly becoming well known as a potent inducer of hepatic enzymes (1–11). As is characteristic of enzyme inducers such as phenobarbital, I increases liver weight, cytochrome P-450 content, aniline hydroxylase, ethylmorphine *N*-demethylase, and cytochrome c reductase but does not increase the microsomal protein per gram of liver content (5).

Ultrastructural changes in rat hepatocytes following I treatment have been extensively examined (12–17). Compound I produced marked proliferation of smooth-surfaced endoplasmic reticulum, with disorganization of the rough endoplasmic reticulum occurring. Most cytoplasmic changes returned to normal within 10 days after the last I administration (15).

The potential for using I to promote detoxification of many xenobiotics exists; and when I is given to female rats several days before a toxicant, it can protect against a variety of substances (3). If I is to be useful in promoting xenobiotic detoxification, the rate of onset of enhanced hepatic drug-metabolizing activity must be determined since the applicability of I lies in its administration after a toxicant has been ingested by a patient.

All cited studies were conducted with female rats. Therefore, it was of interest to learn how quickly I can enhance the activity of the hepatic mixed function oxidase system in male rats and to compare these results with the onset, maximal effects, and duration of induction in female rats.

EXPERIMENTAL

Animal Pretreatment—Male and female rats[1], 120–140 g, were maintained on food[2] and tap water *ad libitum*. To determine the onset of action of I upon hepatic mixed function oxidase activity, both male and female rats were given a single dose of I (20 mg/kg ip) in 0.2% polysorbate 80 in isotonic saline or only the polysorbate 80–saline vehicle and sacrificed at various times after injection.

Hepatic aniline hydroxylase activity (nanomoles of *p*-aminophenol formed per minute per milligram of microsomal protein) and hepatic microsomal cytochrome P-450 content (nanomoles of P-450 per milligram of microsomal protein) were determined in control and experimental animals injected and sacrificed at corresponding times. Three control and three experimental rats were treated and sacrificed at each time point for each sex.

Enzyme, Protein, and Cytochrome P-450 Assays—The 10,000×*g* supernatant fraction was used to determine aniline hydroxylase activity, using an NADPH-generating system (18) and the assay technique of Kato and Gillette (19). Microsomal P-450 was determined by the method of Omura and Sato (20). Microsomal protein was measured by the Folin phenol method (21). These determinations were performed on three pairs of rats sacrificed at 10, 15, 20, 25, 30, 48, 60, and 72 hr after treatment. Averages in corresponding control and experimental groups were compared (Table I).

RESULTS AND DISCUSSION

The effect of a single injection of pregnenolone-16α-carbonitrile (I) at 20 mg/kg on the onset and time course of hepatic aniline hy-

[1] Sasco, Inc., Omaha, Neb.
[2] Purina Lab Chow.

Table I—Hepatic Aniline Hydroxylase Activity and Cytochrome P-450 Content in Male and Female Rats following a Single Dose of I[a]

Time after Treatment, hr	P-450 Content, % of Controls		Aniline Hydroxylase Activity, % of Controls	
	Male	Female	Male	Female
10	99	98	101	102
15	100	109	100	115
20	106	141	130	157
25	113	182	145	163
30	163	170	136	153
48	144	169	122	149
60	128	127	101	130
72	100	101	99	97

[a] Male and female rats were given a single dose of 20 mg/kg ip of I or the vehicle. Three animals from each group of both sexes were sacrificed at the times indicated. Aniline hydroxylase activity was determined as nanomoles of *p*-aminophenol formed per minute per milligram of microsomal protein. Hepatic microsomal cytochrome P-450 was determined as nanomoles of P-450 per milligram of microsomal protein. Each value is the mean of the treated animals over the mean of the control animals times 100.

droxylase activity and cytochrome P-450 content in male and female rats is given in Table I.

As can be seen, aniline hydroxylase activity in the female rats increased sharply at 20 hr after I administration and remained elevated at or above this level until 48 hr posttreatment. In both male and female rats, maximal aniline hydroxylase activity was observed 25 hr after I treatment; the maximum levels achieved in male rats were approximately 89% of those observed in female rats. The difference was not statistically significant due to the small number of animals used. In the male rats, the aniline hydroxylase activity did not remain elevated but began to fall rapidly and had returned to control levels by 60 hr after treatment.

In the female rats, hepatic cytochrome P-450 content was maximal at 25 hr after I treatment and remained elevated until 48 hr, after which it began to decline. In the male rats, peak levels of cytochrome P-450 were observed 30 hr after pretreatment and gradually declined thereafter, reaching control levels by 72 hr after I treatment. As with aniline hydroxylase activity, maximum P-450 levels in male rats were approximately 89% of the values observed in female rats. The difference between the maximum P-450 levels for male and female rats was not significant, although the differences at 20 and 25 hr after I treatment were significant at the $p < 0.05$ level.

Radzialowski (22) investigated the effect of I on bilirubin metabolism in male and female rats. After I pretreatment, the rate of bilirubin metabolism increased 56% in female rats but only 29% in male rats, supporting our general observations. Conney (23) discussed the sex differences involved in the metabolism of various compounds. It appears that in both the induced and uninduced states, female rats metabolize many xenobiotics more rapidly than male rats.

Following a single injection of I at 20 mg/kg, enzyme induction was not seen until at least 20 hr after administration. This time interval might be shortened by using larger doses and/or more frequent administration. Compound I might be useful in situations involving chronic toxicity. Whether I could be used for detoxification following acute toxicity by xenobiotics would depend on the agent and circumstances involved.

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