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Enzyme induction with pregnenolone-16α-carbonitrile—Onset of action and effect of thioacetamide

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RATS PRETREATED with pregnenolone-16α-carbonitrile (PCN) survive ordinarily lethal doses of digitoxin.[1] The mechanism by which PCN protects rats against the toxic manifestations of digitoxin and other poisons may involve induction or activation of the hepatic mixed-function oxidase system. This system detoxifies many xenobiotics by converting them to hydroxylated, readily excretable metabolites,[2] and the activity of this enzymatic system is profoundly increased by PCN pretreatment.[3–5] It is of interest to learn how quickly PCN can enhance the activity of the hepatic mixed-function oxidase system and to determine the rate-limiting step in this enhancement.

All rats used in our experiments were 120–140 g females derived from the Holtzman strain and were obtained from Sasco, Inc., Omaha, Nebr. To determine the onset of action of PCN upon hepatic mixed-function oxidase activity, rats were given either PCN (20 mg/kg, i.p.) or saline–Tween 80 injections as previously described,[5] and sacrificed at various times after injection. Hepatic aniline hydroxylase activity (nmoles *p*-aminophenol formed/min/mg microsomal protein) and hepatic microsomal cytochrome P-450 content (nmoles P-450/mg microsomal protein) were determined in control and experimental animals injected and sacrificed at corresponding times.

The design of the experiment was as follows. Three control and three experimental rats were treated and sacrificed in parallel. Livers were excised and used at once, or immediately transferred to a VirTis freezer maintained at −100°F. Frozen livers were used within a week. Such storage of livers did not significantly affect microsomal protein content, microsomal P-450 content or aniline hydroxylase activity (S. J. Stohs, R. E. Talcott and W. H. Bulger, unpublished observations). Homogenization and subcellular fractionation of livers were performed as previously described.[6] The 10,000 *g* supernatant fraction was used to determine aniline hydroxylase activity using an NADPH-generating system,[7] and the assay technique of Kato and Gillette.[8] Microsomal P-450 was determined by the method of Omura and Sato.[9] Microsomal protein was measured by the method of Lowry *et al.*[10] These determinations were performed on three pair of rats sacrificed at 3, 6, 10, 20, 24, 48, 60 and 72 hr after treatment and on six pair of rats sacrificed at 13 and 16 hr after treatment. Averages in corresponding control and experimental groups were compared and the results of this study are depicted in Fig. 1.

These results demonstrate that a single intraperitoneal dose of PCN at 20 mg/kg increases aniline hydroxylase activity and cytochrome P-450 content in parallel. Significant increases were first noted in PCN-treated rats sacrificed 16 hr after treatment. These increases were maximal in rats sacrificed 24–48 hr after PCN treatment. Animals sacrificed 72 hr after PCN treatment had control levels of hepatic P-450 and aniline hydroxylase (Fig. 1).

These results suggest that the PCN-induced accumulation of cytochrome P-450 is coupled to the PCN-induced increase in aniline hydroxylase activity. To further examine this possibility, and to compare PCN induction to phenobarbital induction, we studied the ability of thioacetamide, an inhibitor of protein synthesis, to block the effects of phenobarbital and PCN on hepatic microsomal P-450 levels and aniline hydroxylase activity.

Six groups of four to six rats were placed in separate cages. Rats received i.p. injections of either PCN (20 mg/kg), phenobarbital (100 mg/kg), thioacetamide (50 mg/kg), phenobarbital (100 mg/kg) plus thioacetamide (50 mg/kg), PCN (20 mg/kg) plus thioacetamide (50 mg/kg) or the saline–Tween 80 vehicle. In each case, the injection volume was 1·0 ml and injections were given once on each of 3 successive days. Rats were fasted after the third dose and sacrificed 16 hr later. Livers were excised and immediately placed in a −100 F VirTis freezer. Each liver was assayed for aniline hydroxylase activity, microsomal P-450 and microsomal protein as described above. Results were averaged and groups were compared using Student's *t*-test (Table 1).

Thioacetamide-pretreated rats manifested lower microsomal protein levels, and proportionately lower aniline hydroxylase and cytochrome P-450 levels, when compared to control rats. Thus, on a per mg of microsomal protein basis, aniline hydroxylase and P-450 levels are similar in control and thioacetamide-pretreated rats (Table 1). As previously reported,[3] PCN, unlike phenobarbital, increases aniline hydroxylase activity and microsomal P-450 content without increasing microsomal protein content (Table 1). Thioacetamide, given concurrently with PCN or phenobarbital, greatly inhibited the accumulation of P-450 and entirely blocked the increase in aniline hydroxylase activity (Table 1).

Sladek and Mannering[11] reported that thioacetamide could block phenobarbital induction of drug metabolism but not polycyclic hydrocarbon induction. Our findings that thioacetamide can also block PCN induction suggest that PCN induction is similar to phenobarbital induction rather than polycyclic

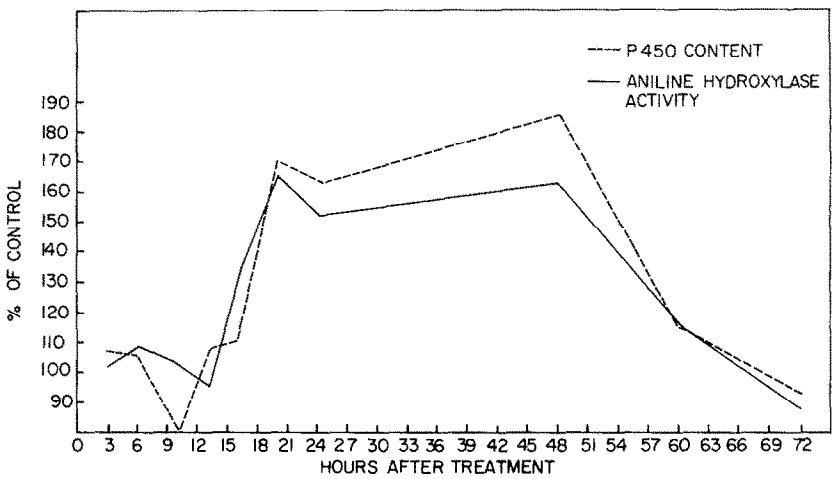

FIG. 1. Effect of PCN on aniline hydroxylation and cytochrome P-450 levels. Groups of three to six rats were injected and then sacrificed at 3, 6, 10, 13, 16, 20, 24, 48, 60 or 72 hr later. Hepatic aniline hydroxylase activity and cytochrome P-450 content were determined and compared with control groups sacrificed at corresponding times.

hydrocarbon induction. Other evidence supports the view that PCN induction and phenobarbital induction are similar. Microsomes from PCN and phenobarbital-pretreated animals showed similar catalytic activity toward steroid hormones,[4] bile acids,[4] sterols[4] and cardenolides.[5] Also, the carbon monoxide-binding hemoprotein in microsomes from PCN-pretreated animals is spectrally similar to the P-450 in microsomes from control and phenobarbital-pretreated animals.[3] In contrast, polycyclic hydrocarbons induced the synthesis of a spectrally and catalytically distinct hemoprotein, "cytochrome P-448."[12,13]

Although microsomes from PCN-pretreated rats and phenobarbital-pretreated rats have properties in common, differences in *N*-demethylase activity have been noted. Lu *et al.*[3] found that phenobarbital preferentially stimulated benzphetamine *N*-demethylation, but that PCN preferentially stimulated ethylmorphine *N*-demethylation. Possibly, the hemoproteins induced by phenobarbital and PCN differ, although

TABLE 1. EFFECT OF THIOACETAMIDE ON PHENOBARBITAL AND PCN INDUCTION*

Pretreatment group	Microsomal protein (mg/g liver)	Aniline hydroxylase (nmoles *p*-aminophenol/min/mg microsomal protein)	P-450 (nmoles/mg microsomal protein)
Saline (6)	17·1 ± 0·84	0·541 ± 0·027	0·41 ± 0·036
Phenobarbital (6) (100 mg/kg)	27·8 ± 1·38†	0·737 ± 0·056†	1·02 ± 0·110†
PCN (4) (20 mg/kg)	19·1 ± 1·66	0·807 ± 0·062†	0·89 ± 0·046†
Thioacetamide (6) (50 mg/kg)	12·5 ± 1·09‡	0·535 ± 0·106	0·42 ± 0·070
Phenobarbital plus thioacetamide (6)	17·4 ± 1·48	0·512 ± 0·048§	0·58 ± 0·065‡,§
PCN plus thioacetamide (4)	17·3 ± 0·59	0·554 ± 0·034‖	0·591 ± 0·078‡,‖

* Animals received the treatments indicated above on each of 3 successive days. Numbers in parentheses are the numbers of animals in each group. Animals were fasted for 16 hr after the last dose and then sacrificed. Livers were excised, frozen and later assayed for microsomal protein, aniline hydroxylase and cytochrome P-450 by the methods of Lowry *et al.*,[10] Kato and Gillette,[8] and Omura and Sato[9] respectively. Results are expressed as the mean ± S. E.

† $P < 0.01$ with respect to saline-treated animals.

‡ $P < 0.05$ with respect to saline-treated animals.

§ $P < 0.01$ with respect to phenobarbital-treated animals.

‖ $P < 0.01$ with respect to PCN-treated animals.

such differences have not yet been revealed by spectral studies. Alternatively, phenobarbital and PCN may differentially affect other components of the mixed-function oxidase system or its microenvironment.

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Selectivity of pentylenetetrazol on brain monoamine metabolism

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IT IS WELL established that drugs which lower monoamine concentrations in brain tissue lower seizure threshold, and drugs which increase these levels increase threshold.[1] These data have suggested that a relationship exists between brain monoamine metabolism and seizure activity. To investigate this, we have studied the effects of pentylenetetrazol alone and in conjunction with seizures on brain monoamine metabolism. We have estimated the effects of pentylenetetrazol on the metabolism of monoamines in feline brain tissue *in vivo* after doses which did little to change behaviour and after those which produced frank clonic convulsions. Because changes in monoamine metabolite concentrations in cerebrospinal fluid (CSF)[2–5] are thought to reflect changes in central monoamine-mediated activity, we have examined these metabolites in serial samples of cisternal cerebrospinal fluid of the cat.

Cats were caged individually in a room maintained at 23–24° with lights on from 7:00 a.m. to 7:00 p.m. They were observed, and rectal temperature was monitored from 9.00 a.m. to 6.00 p.m. during control and experimental periods. Cats were divided into four groups and cannulas were implanted[6] into the cisterna magna which permitted repeated sampling of CSF from the unanesthetized animal. We assayed[7,8] for the major brain metabolites of 5-hydroxytryptamine and dopamine, 5-hydroxyindolacetic acid (5-HIAA) and homovanillic acid (HVA), respectively, which exit from brain via CSF.[5] During a control period, 1·0 ml samples of CSF were withdrawn (four to five/day) at intervals not less than 2 hr. Experimental samples were withdrawn at the same times of day used in the control period and the percentage change from control absolute concentration was individually determined for each cat. Thus, each cat served as his own control. The mean and variation of the experimental samples for each group and for similar times of day