Effects of the Porphyrogenic Agent 2-Allyl-2-isopropylacetamide on the Hydroxylation of Testosterone in Rat Liver Microsomal Fraction

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In rats 2-allyl-2-isopropylacetamide causes a porphyria with many similarities to acute intermittent porphyria in man (Goldberg & Rimington, 1955; Tschudy & Bonkowsky, 1972). The finding that patients suffering from acute intermittent porphyria may have abnormal steroid-excretion patterns (Goldberg et al., 1969; Kappas et al., 1971) prompted an investigation of the effects of 2-allyl-2-isopropylacetamide on steroid metabolism in the rat. Reports that 2-allyl-2-isopropylacetamide causes a decrease of microsomal cytochrome P-450 (De Matteis, 1971) suggested a study of its effect on steroid hydroxylation.

Male albino rats 10-12 weeks old were injected with 2-allyl-2-isopropylacetamide (300mg/kg body wt.). They were killed and liver microsomal fractions were isolated for measurement of testosterone hydroxylation and cytochrome P-450 concentration. [4-14C]Testosterone was the substrate for hydroxylation, the incubation system being a modification of that described by Conney et al. (1968). 16α-, 6β-, 2β- and 7α-hydroxylated metabolites were isolated by t.l.c. and determined by liquid-scintillation counting of their radioactivity. The identity of the hydroxylated metabolites was established by t.l.c. and g.l.c. of the trimethylsilyl derivatives (Demisch & Staib, 1969). Cytochrome P-450 was determined by the method of Omura & Sato (1964).

At 14h after a single injection of 2-allyl-2-isopropylacetamide the 16α-, 6β- and 2β-hydroxylase activities were decreased to respectively 44, 46 and 39% of control values; 7α-hydroxylase activity was unaffected (Table 1). Accompanying the decrease in hydroxylation there was a fall in microsomal cytochrome P-450 concentration. Treatment of rats for 3 days with 2-allyl-2-isopropylacetamide gave similar results, but this time there was a small, though statistically significant, decrease in 7α-hydroxylation. 2-Allyl-2-isopropylacetamide (10.0μmol; 4.0mmol/l) added to enzyme incubation mixtures caused some inhibition of testosterone hydroxylation (10-20%). However, preincubation of liver homogenates with 2-allyl-2-isopropyl[2-14C]acetamide (0.11mmol; 11.0mmol/l) followed by isolation of the microsomal fractions showed that very little labelled 2-allyl-2-isopropylacetamide or its metabolites remained bound to the microsomal fragments (equivalent to less than 0.02μmol of 2-allyl-2-isopropylacetamide added to each hydroxylase incubation mixture compared with 0.7μmol of [4-14C]-testosterone). A similar result was obtained when liver homogenates were preincubated with 2-allyl-2-isopropylacetamide and NADPH. It therefore appears that injected 2-allyl-2-isopropylacetamide did not act directly as an enzyme inhibitor but caused a decrease in microsomal hydroxylase content. Microsomal fractions from the livers of 2-allyl-2-isopropylacetamide-treated rats showed the green discolouration that has been attributed to the presence of unusual degradation products of microsomal haem, arising from the breakdown of cytochrome P-450 (De Matteis, 1971).

The results suggest that rats with 2-allyl-2-isopropylacetamide-induced porphyria will have an abnormal steroid metabolism. Whether this would lead to increased excretion of 3β-hydroxylated or 5β-reduced metabolites, as found in humans with acute intermittent porphyria, remains to be seen. However, in view of the possibility that induction of porphyrin biosynthesis by 2-allyl-2-isopropylacetamide may be related to its effects on cytochrome P-450 (Satyanaryana Rao et al., 1972) and therefore on steroid hydroxylation, and that similarly acting unidentified factors may be involved in...
Adult male rats were injected once with 2-allyl-2-isopropylacetamide (300mg/kg body wt.) or given three injections over 3 days. Control groups received an equivalent volume of 0.9% NaCl. Rats were killed 14h after the last injection and livers were removed for assay of testosterone hydroxylase activity and cytochrome P-450. Results are means ± s.e.m., six animals being in each group. Significance of difference from corresponding 0.9% NaCl-treated control group: ***P < 0.001; **P < 0.005; *P < 0.01.

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<tr>
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<th>Testosterone hydroxylation</th>
<th>Cytochrome P-450 content</th>
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<tr>
<td></td>
<td>(nmol of product formed/min per mg of microsomal protein)</td>
<td>(nmol/mg of microsomal protein)</td>
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<td></td>
<td>Total hydroxy-metabolites</td>
<td>16α-Hydroxy-testosterone</td>
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<tr>
<td>0.9% NaCl, one injection</td>
<td>11.21 ± 1.21</td>
<td>4.09 ± 0.68</td>
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<tr>
<td>2-Allyl-2-isopropylacetamide, one injection</td>
<td>4.00 ± 1.64***</td>
<td>1.79 ± 0.86***</td>
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<tr>
<td>0.9% NaCl, three injections</td>
<td>10.57 ± 2.14</td>
<td>3.73 ± 1.05</td>
</tr>
<tr>
<td>2-Allyl-2-isopropylacetamide, three injections</td>
<td>3.71 ± 0.86***</td>
<td>0.94 ± 0.39***</td>
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human acute porphyria, it would be of interest to know if there is a decreased production of hydroxylated steroid metabolites in humans with this disease.

The effect of 2-allyl-2-isopropylacetamide on the 16α-, 6β- and 2β-hydroxylations and the relative lack of effect on 7α-hydroxylation is in accord with the view that these reactions may be catalysed by separate hydroxylase systems (Conney et al., 1968; Jacobson & Kuntzman, 1969; Levin & Kuntzman, 1969). A possible explanation of the present results is that the different forms of microsomal cytochrome (P-450 and P-448) thought to be active in the different hydroxylation reactions (Lu et al., 1971, 1972) may have differing susceptibilities to the effects of 2-allyl-2-isopropylacetamide.

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The Effects of Aflatoxin B₁ on the Hepatic Structure and Ribonucleic Acid Synthesis in Rats Fed on a Marginally Choline-Deficient Diet

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Rogers & Newberne (1971) have reported that feeding rats on a marginally choline-deficient diet has a protective action against the acute toxicity of aflatoxin B₁, without a comparable effect on the hepatocarcinogenic action of the toxin. This raises the possibility of separating, both from a morphological and a biochemical viewpoint, those changes that are associated with the toxic action and those associated with the carcinogenic activity. We have confirmed the protective action of the diet against the acutely toxic effect of aflatoxin B₁, and find that this is accompanied by a drastic diminution in the necrogenic action of the toxin. The rapid inhibition of hepatic RNA synthesis that is induced by the toxin is found to be largely unaffected by the diet, and, in agreement with this, nucleolar segregation occurs in the hepatic nuclei throughout the lobule of both control and diet-fed animals. It is tentatively concluded that the acutely hepatotoxic action of aflatoxin B₁ involving cell necrosis is not related to the inhibition of RNA synthesis.


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