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Effects of indomethacin administration on hepatic steroid and drug metabolism in male and female rats

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The broad spectrum of biological effects of prostaglandins has made them the subject of intensive investigation in many laboratories. Inhibitors of prostaglandin synthesis, such as indomethacin, represent powerful tools for studying the actions of prostaglandins [1, 2]. However, care must be taken when administering prostaglandin inhibitors to experimental animals because of their toxic effects at high doses [3, 4]. The liver, for example, has been shown to undergo degenerative changes as a result of long-term indomethacin administration to rats [3]. Nonetheless, relatively large amounts of indomethacin have been given to animals in attempts to determine the role of prostaglandins in hormone action [5–10] and in other types of physiological and pharmacological research. Since the liver is a major site of hormone metabolism, changes in hepatic function after indomethacin administration may complicate interpretation of changes in plasma hormone concentrations and/or hormone action. Furthermore, since hepatic microsomal enzymes are of principal importance in oxidative drug metabolism, effects of indomethacin on the liver might alter the half-life of, and consequently the response to, other substances simultaneously administered. For these reasons, it is important to know what effects, if any, indomethacin has on hepatic metabolism. The data presented in this report indicate that indomethacin administration can have profound effects on both drug and steroid metabolism by hepatic microsomal enzymes.

METHODS

Male and female Sprague–Dawley rats, 50 to 60-days-old, were obtained from Zivic–Miller Laboratories, Pittsburgh, PA, and maintained under standardized conditions of light (6:00 a.m. to 6:00 p.m.) and temperature (22°) on a diet of Purina Laboratory Chow and water *ad lib.* Indomethacin (5 mg/kg) was administered as an intraperitoneal injection in 0.1 M phosphate buffer (pH 8.0) twice a day for 1, 2 or 3 days. The start of treatment was staggered, permitting control and experimental animals in each group to be killed on the same day. All animals received the same number of injections of indomethacin or vehicle only, as appropriate.

Animals were killed by decapitation between 9:00 and 10:00 a.m. and livers were removed quickly and homogenized in cold 1.15% potassium chloride. Homogenates were centrifuged at 9000*g* for 20 min in a Sorvall refrigerated centrifuge. Aliquots of the supernatant fraction were removed for enzyme assays and the remainder was centrifuged at 105,000*g* for 60 min in a Beckman preparative ultracentrifuge. All steps in the preparation of microsomes were performed with the tissue kept at 0–4°. Microsomal pellets were resuspended immediately prior to use in 1.15% potassium chloride containing 0.05 M-Tris–HCl (pH 7.4) at a concentration of 3–4 mg protein/ml. Microsomal cytochrome P-450 was measured as described by Omura and Sato [11] and protein was measured by the method of Lowry *et al.* [12]. The demethylation of ethylmorphine and the hydroxylation of aniline were assayed as the rates of formation of formaldehyde [13] or *para*-aminophenol [14], respectively, by 0.5 ml liver 9000 *g* supernatant (equivalent to 200 mg/ml) incubated with glucose 6-phosphate (9.0 μmoles), $MgSO_4$ (24.2 μmoles), Tris–HCl (0.05 M, pH 7.4) and ethylmorphine–HCl (12 μmoles) or aniline–HCl (6 μmoles) in a final volume of 3.0 ml. Semicarbazide–HCl (25 μmoles) served as a trapping agent for formaldehyde produced from ethylmorphine.

Hepatic Δ^4-steroid hydrogenase activity was measured as described by Tompkins [15] using the 9000 *g* supernatant fraction as enzyme source and corticosterone as substrate. Hepatic supernatant fraction equivalent to 10 mg tissue was incubated with 150 ηmoles corticos-

Table 1. Effects of indomethacin administration on hepatic drug and steroid metabolism in female rats*

Days treated	Microsomal protein (mg/g liver)	Ethylmorphine demethylase (nmoles/min/g liver)	Aniline hydroxylase (nmoles/min/g liver)	Cytochrome P-450 (nmoles/mg protein)	Δ^4-Steroid hydrogenase (nmoles/min/g liver)	Corticosterone half-life (min)
0	29.3 ± 2.0	136.4 ± 8.8	22.0 ± 2.9	0.65 ± 0.07	653.2 ± 58.7	10.9 ± 0.8
1	27.4 ± 1.9	91.4 ± 7.6†	11.6 ± 0.9†	0.41 ± 0.02†	449.3 ± 27.5†	15.4 ± 1.1†
2	27.2 ± 1.8	55.1 ± 5.1†	6.5 ± 0.5†	0.32 ± 0.02†	317.2 ± 28.4†	19.3 ± 1.4†
3	25.2 ± 1.9	33.9 ± 4.0†	3.1 ± 0.4†	0.25 ± 0.02†	294.1 ± 24.9†	22.0 ± 1.7†

*Values are expressed as mean ± S.E.; five to eight values per group.
†$P < 0.05$ (vs controls).

Table 2. Effects of indomethacin administration on hepatic drug and steroid metabolism in male rats*

Days treated	Microsomal protein (mg/g liver)	Ethylmorphine demethylase (nmoles/min/g liver)	Aniline hydroxylase (nmoles/min/g liver)	Cytochrome P-450 (nmoles/mg protein)	Δ^4-Steroid hydrogenase (nmoles/min/g liver)	Corticosterone half-life (min)
0	36.2 ± 1.8	486 ± 25	28.0 ± 1.5	0.77 ± 0.05	125.3 ± 8.1	17.8 ± 1.1
1	36.9 ± 1.5	425 ± 31	24.9 ± 1.9	0.68 ± 0.06	112.1 ± 6.8	19.0 ± 1.3
2	37.9 ± 1.9	333 ± 26†	19.8 ± 1.2†	0.56 ± 0.05†	99.2 ± 7.3†	20.8 ± 1.9
3	35.9 ± 1.1	219 ± 17†	13.9 ± 1.0†	0.44 ± 0.05†	88.7 ± 6.9†	24.9 ± 2.0†

*Values are expressed as the mean ± S.E.; five to eight values per group.
†$P < 0.05$ (vs controls).

terone for 15 min under air in a total volume of 0.25 ml. After incubation, steroids were extracted with chloroform and ring A reduction (Δ^4-hydrogenase activity) was evaluated using the *p*-nitrophenyl hydrazine reaction [16] and u.v. absorption at 240 nm. The *in vivo* plasma half-life of corticosterone was determined using plasma samples obtained 5, 10, 15 and 20 min after the intravenous injection of 1.0 μCi[1,2-^{3}H]corticosterone (sp. act. 50 Ci/m-mole), as described previously [17]. Radioactivity present in 100-μl aliquots of plasma was determined using a Packard Tri Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Enzyme activities in Tables 1 and 2 are expressed as nmoles product formed or substrate metabolized per min/per g of liver. However, since indomethacin did not significantly affect microsomal protein concentration, the effects observed are equally valid when expressed per mg of microsomal protein. In female rats, within 24 hr after starting indomethacin treatment, the rates of ethylmorphine demethylation and aniline hydroxylation were reduced significantly (Table 1). With continued administration of the drug, enzyme activities were decreased further. The decline in drug metabolism was accompanied by a progressive fall in the hepatic microsomal concentration of cytochrome P-450, the terminal oxidase for ethylmorphine and aniline metabolism. However, enzyme activities decreased proportionally to a greater extent than cytochrome P-450 content. In male rats (Table 2), the effects of indomethacin on drug metabolism were qualitatively similar to those in females, but were considerably smaller in magnitude. Reinicke and Klinger [18] previously reported that a slightly smaller dose of indomethacin (6 mg per kg per day for 3 days), when given to phenobarbital-treated male rats, prolonged hexobarbital sleeping time, suggesting inhibition of hepatic hexobarbital metabolism. However, neither cytochrome P-450 concentrations nor microsomal metabolism of hexobarbital was determined in those studies.

Indomethacin treatment also produced a significant decrease in the activity of hepatic steroid Δ^4-hydrogenase (Tables 1 and 2), the rate-limiting step in corticosteroid metabolism [19, 20]. Accordingly, the rate of disappearance of corticosterone from plasma decreased with increasing duration of indomethacin administration. The effects of indomethacin on steroid metabolism were also greater in females than males. These observations indicate that doses of indomethacin used occasionally in studying prostaglandin actions can have profound effects on hepatic drug- and steroid-metabolizing enzymes. Such effects may alter the biological activity of other drugs being administered simultaneously and may modify plasma steroid concentrations independently of changes in endocrine secretion. Interpretation of apparent sex differences in drug or hormone action is further complicated by the greater potency of indomethacin in female rats than in males. Changes in hepatic function, therefore, must be considered when evaluating the effects of indomethacin administration of experimental animals for even relatively short periods of time.

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Influence of calcium ethylenediaminetetraacetate on the metabolism of collagen and noncollagen proteins of carrageenin granuloma in rats

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A number of agents, including steroids, have been used in attempts to alter inflammatory responses. Granulation tissue formed in the course of chronic inflammation contains a large amount of collagen in its extracellular space. Thus, agents affecting protein metabolism may be important in regulating the formation and resorption of