in the hypothalamus and pituitary levels of approximately 4 ng/g. Our data demonstrate that the catechol metabolites of estradiol and estrone have tissue concentrations at least ten times those of their parent compounds. The higher levels of 2-hydroxy estrogen might be explained by the marked binding of catechol estrogens to tissue proteins (*18*). The relatively high concentration of catechol estrogens in brain may also be a result of synthesis within the central nervous system (*19*). Recent studies in our laboratory (*20*) have confirmed the presence of a catechol estrogen–forming enzyme in brain that has activity comparable to that found in liver. Nevertheless, the results presented here, coupled with the recent reports demonstrating a potent competition by catechol estrogens for estrogen receptors of the pituitary and hypothalamus in vitro (*4, 21*) suggest that this last finding may be of physiological significance. In addition, the administration of catechol estrogens intracerebrally to ovariectomized guinea pigs (*3*) or peripherally to immature male rats (*5*) and estrogen-primed female rats (*6*) has been shown to have profound effects on the circulating levels of plasma luteinizing hormone. These responses were similar to (*6*) or greater than (*3, 5*) those observed with the estrogens themselves and again suggest that the catechol estrogens are acting at the level of the pituitary or hypothalamus. The data presented here establish the catechol estrogens as major estrogenic constituents of brain and various endocrine tissues, occurring in concentrations that exceed those of their parent compounds. Taken together with their biological actions these results indicate that the catechol estrogens may have an important role in neuroendocrine regulation.

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11. The COMT was prepared according to the method of J. Axelrod and R. Tomchick [*J. Biol. Chem.* **233**, 702 (1958)] as modified by B. Nikodejevic, S. Senoh, J. W. Daly, and C. R. Creveling [*J. Pharmacol. Exp. Ther.* **174**, 83 (1970)].
12. In areas of high norepinephrine content (that is, hypothalamus) the organic phase was washed once with 0.5 ml of 0.1*N* HCl to remove any counts of radioactivity that might be due to very slight extraction of ^{3}H-labeled normetanephrine (*10*). This step was found to be unnecessary, however, for all other tissues assayed.
13. Thin-layer chromatography was performed on Silica Gel 125 mm 60 F-254 precoated plates (Merck). The solvent systems used were, by volume (i) chloroform, methanol, acetic acid, 96 : 3 : 1; (ii) chloroform, ethanol, 95 : 5; (iii) benzene, ethanol, 3 : 1; (iv) ethyl acetate, hexane, acetic acid, ethanol, 72 : 13.5 : 10 : 4.5; (v) ethyl acetate, cyclohexane, 50 : 50. After development and drying of the plates, 1^{-2}-cm sections of the silica were scraped into counting vials containing 2 ml of ethanol. After agitation for 30 seconds, 10 ml of Aquasol was added and the vials were counted. Radioactivity cochromatographed with the four nonradioactive standards (Fig. 1). Bidimensional chromatography on Silica Gel was performed by first developing the plates in solvent system (i). After being dried at room temperature the plates were rotated 90° and developed again in either solvent system (i) or (v). Radioactivity again cochromatographed with the nonradioactive standards.
14. The reaction products from several hypothalamic extracts were purified by thin-layer chromatography. After elution from the silica they were subjected to mass spectral analysis by direct insertion probe (VG Micro Mass 16 F; accelerating voltage, 4 kv; electron energy, 70 ev; ionization current, 100 μa; source temperature, 200°C). The mass spectra showed an intense molecular ion at *m/e* 300, and other ions characteristic of monomethylated derivatives of 2-hydroxy estrone (*m/e* 285, 215, 202, 176). This confirms the presence of methylated catechol estrogens in the thin-layer band being analyzed.
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3 November 1976; revised 3 March 1977

Porphyrin Induction: Equivalent Effects of 5α-H and 5β-H Steroids in Chick Embryo Liver Cells

Abstract. *The 5α-H (A : B* trans*) and 5β-H (A : B* cis*) steroids are equipotent in inducing δ-aminolevulinic acid synthetase and porphyrin accumulation in chick embryo liver cells maintained in serum-free culture medium. Thus there is no specific steric requirement for porphyrin-inducing activity in steroids.*

We have found that 5α-H steroids are at least as potent in inducing δ-aminolevulinic acid synthetase (ALA-synthetase; E.C.2.3.1.37) and porphyrin accumulation as 5β-H steroids in chick embryo liver cells maintained in serum-free medium. Our findings do not support the idea that there is a specific steric requirement for porphyrin-inducing activity in steroids (*1*). Moreover, our findings suggest that in patients with acute intermittent porphyria the diminished hepatic steroid Δ^4-5α-reductase activity that leads to the accumulation of 5β-H steroids is not related to the pathogenesis of the disease (*2, 3*).

We obtained the steroids from Sigma and checked their purity by determining their melting points; the melting points corresponded to accepted values (*4*). Chick embryo liver cells were maintained in serum-free culture medium containing insulin and thyroxine according to methods described (*5*). For measurement of steroid-induced porphyrin accumulation, livers from 18-day-old chick embryos were dispersed with a mixture of collagenase and hyaluronidase and maintained on the surface of plastic petri dishes (60 mm in diameter) in a medium containing 5 ml of Waymouth MD 705/1; each liter of Waymouth medium was supplemented with 60 mg of penicillin G, 100 mg of streptomycin sulfate, 1.0 mg of insulin, and 1.0 mg of thyroxine. For measurement of steroid-induced ALA-synthetase accumulation, cells were maintained on the surface of 100-mm petri dishes containing 15 ml of the above medium. After an initial incubation of 24 hours at 37°C in 5 percent CO_2 in air, the medium was discarded and replaced with fresh medium, and the dishes were incubated for a further 24 hours. Steroids were dissolved in 10 μl of 95 percent ethanol for addition to 60-mm dishes and in 30 μl of 95 percent ethanol for addition to 100-mm dishes, and were added during the second 24-hour incubation period only.

Porphyrins and ALA-synthetase were assayed as described (*6*). To assay ALA-synthetase, we pooled the cells from two 100-mm diameter dishes in order to obtain sufficient material for the assay. The activity of the steroids was studied at

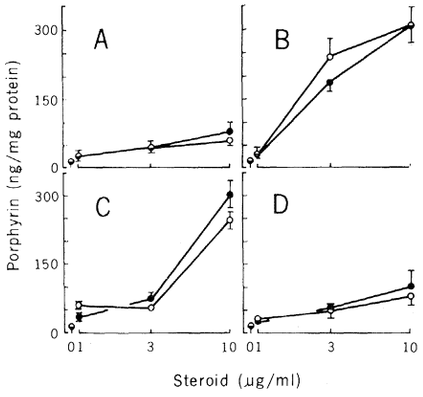

Fig. 1. Porphyrin accumulation in the cells and medium 24 hours after the administration of the 5α (•) and 5β (○) steroid epimers. (A) Pregnane-3,20-dione. (B) Androstane-3α-ol-17-one. (C) Androstane-3,17-dione. (D) Pregnane-3β-ol-20-one. The points represent the mean of at least four determinations ± standard error.

concentrations of 1, 3, and 10 μg/ml for porphyrin accumulation and at 3, 10, and 30 μg/ml for ALA-synthetase activity.

The porphyrin-inducing activities of 5α-pregnane-3,20-dione and of 5α-androstane-3α-ol-17-one do not differ from those of their respective epimers (Fig. 1, A and B) at any of the doses tested. This result is in contrast to the studies of Granick and Kappas (*1*), who reported a ratio of inducing activity for 5βH to 5αH as 4:1 with the pregnane epimers and of 5:1 with the androstane epimers. A possible reason for this difference is that we measured porphyrins quantitatively, whereas Granick and Kappas used a qualitative technique; moreover, we compared the activity of 5αH and 5βH epimers at a range of doses while it is not clear whether they used more than one dose to compare activities. In our study we used a serum-free medium to avoid possible interference by endogenous hormones in the serum and the possible complication of differential binding of epimers to serum proteins. The porphyrin-inducing activities of 5α-androstane-3,17-dione and of 5α-pregnane-3β-ol-20-one do not differ from those of their respective epimers (Fig. 1, C and D), thus strengthening the conclusion that there is no difference between the porphyrin-inducing activity of 5αH and 5βH epimers. Gidari *et al.* (*7*) showed that cyproterone, a synthetic steroid possessing neither a 5αH nor a 5βH configuration, was a highly potent inducer of ALA-synthetase in chick embryo liver cells in culture. For this reason we studied the activity of Δ^4-androstene-3,17-dione and Δ^5-pregnane-3β-ol-20-one; both steroids showed considerable activity.

The ALA-synthetase–inducing activities of 5α-pregnane-3,20-dione, 5α-androstane-3α-ol-17-one, 5α-pregnane-3β-ol-20-one, and 5α-androstane-3,17-dione do not differ from those of their respective epimers except in the case of the pregnane epimers (Fig. 2, A and C) at doses of 30 μg/ml. The β-pregnane epimers are devoid of activity at this dose because of a direct toxicity on the chick embryo liver cells in agreement with previous findings (*1*). This finding corresponds essentially with the results of Edwards and Elliott (*8*) who measured the ALA-synthetase–inducing activity of these steroids in isolated rat liver cells and concluded that steroids with a 5αH structure are at least as active as the corresponding 5βH epimers. Edwards and Elliott (*8*) pointed out that their results were in conflict with those previously reported in chick embryo liver cells and it appeared that a species difference existed in response to these steroids. Our present findings and those of Edwards and Elliott show clearly that there is no steric requirement for activity in steroids. A possible explanation for the difference in our present results and those obtained by Granick and Kappas (*1*) is the fact that we utilized medium devoid of protein. It is well known that the degree of steroid hormone binding to plasma proteins can regulate the supply of hormone to a particular cell and thus influence biological activity (*9*). It is therefore possible that in the study of Granick and Kappas (*1*) the 5αH steroids may have been bound more strongly to serum proteins than 5βH steroids, thus explaining the apparently lower activity.

The idea that 5βH steroid metabolites are more potent than 5αH metabolites led to the suggestion that patients with acute intermittent porphyria (AIP) might have abnormalities in steroid hormone biotransformation leading to formation of excessive amounts of 5β metabolites from precursor steroids (*2*). This idea received further support from the finding in AIP patients that there is a marked deficiency in hepatic Δ^4-5α-reductase and that this leads to the formation of an increased ratio of 5βH to 5αH metabolites (*2*, *3*). The suggestion that the defect in 5α-reductase and the relative increase in 5βH compounds may be of importance in the induction of ALA-synthetase in AIP has been criticized by Schmid (*10*) and Pimstone (*11*) because of the equal potency of 5αH and 5βH steroids in rat liver cells and the existence of a group of related individuals with a genetic defect in 5α-reductase activity who do not suffer from porphyria. Our results showing that 5βH steroids do not possess higher potency than 5αH steroids remove the

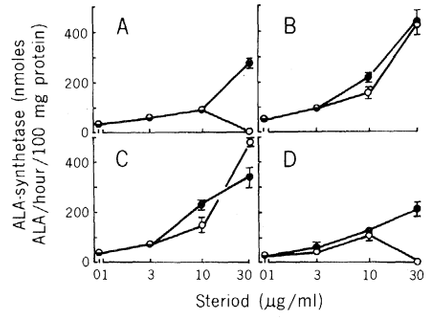

Fig. 2. δ-Aminolevulinic acid-synthetase activity in the cells 24 hours after the administration of the 5α (•) and 5β (○) steroid epimers. (A) Pregnane-3,20-dione. (B) Androstane-3β-ol-17-one. (C) Androstane-3,17-dione. (D) Pregnane-3β-ol-20-one. The points represent the mean of at least four determinations ± standard error.

essential basis for the hypothesis that the 5α-reductase defect is a key factor in the pathogenesis of AIP. Our previous studies on porphyrin-inducing drugs led us to suggest that three factors are important for porphyrin-inducing activity: lipid solubility (lipophilicity), resistance to metabolism to compounds of lower lipophilicity, and the possession of a group capable of participating as an electron donor in hydrogen bond formation (*12*). It is possible that these too are critical factors in the porphyrin-inducing activity of steroids.

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13. Supported by the Medical Research Council of Canada.

22 March 1977