the hypocenters of great shallow-thrust earthquakes are located near the deepest, most landward extent of their rupture zones (*2*). These hypocenters are only a few tens of kilometers from regions of possible magma generation. Therefore, deformation in the hypocentral region may affect the production or rise of magma from its source region. Dilatancy in the hypocentral region and diffusion of a vapor phase from the region of magma generation is a more speculative cause.

The concept of interrelated seismic and volcanic activity cannot yet be evaluated everywhere. A systematic examination of the occurrence parameters of great earthquakes is not yet available for many regions (Indonesia and most of the southwestern Pacific island arcs). In other regions volcano catalogs either do not exist (Aleutian arc) or do not extend back more than a few decades (most of the southwestern Pacific).

In many areas there is no clear association of volcanic and seismic activity. A few segments of convergent plate margins have neither large earthquakes nor active volcanoes (northern Peru–southern Ecuador, Tonga from 23°S to 28°S) (*10*). Many segments have active volcanoes but infrequent large earthquakes (northern Ryukyus, Izu-Bonin-Marianas, Indonesia from 106°E to 122°E) (*10*). Other segments have great earthquakes but little or no recent reported volcanic activity (Nankai Trough, Peru, Mexico, southern Kuriles, southern Ryukyus). Northern Honshu and Ecuador have both large earthquakes and active volcanoes but no obvious volcanic sequence related to great shallow earthquakes, perhaps because the rupture zones in these areas are a great distance from the volcanic chain.

Qualitative forecasts of varying levels of reliability can be made from the associations described here. It is fairly certain that in Central America volcanic activity near the rupture zones of future large shallow-thrust earthquakes will be unusually high for about a decade after the earthquakes. In the past the two largest eruptions in Central American history (Cosigüina in 1835 and Santa Maria in 1902) occurred within months after nearby great earthquakes.

The pattern of volcanic activity can suggest regions that might be preparing for a large shallow-thrust earthquake and therefore deserve careful study. Two such regions are suggested here. One is central and western El Salvador in Central America (Fig. 1), which is a prominent seismic gap (*7*). The volcanoes of this region have been dormant since 1955 with the exception of one small eruption in 1966. Before 1955 there was essentially continuous volcanic activity in this region. The other region deserving attention is the Guadeloupe arc of the northern Antilles (Fig. 4), where the current pattern of volcanic activity is very similar to the volcanic activity which preceded the large earthquake of 1897.

The crude method of evaluating volcanic activity used here probably masks some relations between volcanic and seismic activity. For example, it does not reveal the relation between earthquakes and volcanic activity in the Sagami Trough region of Japan which is made clear only by a detailed study of the eruption pattern of a particular volcano in that region (*5*). Such detailed studies of active volcanoes may have great value in the long-range forecasting of large shallow earthquakes.

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Catechol Estrogens: Presence in Brain and Endocrine Tissues

Abstract. *Catechol estrogens have been identified and measured in rat brain and various endocrine tissues with the use of a sensitive radioenzymatic assay. The specificity of this assay was confirmed by thin-layer chromatography and mass spectral analysis of the reaction products. The concentration of catechol estrogens in the hypothalamus and pituitary are at least ten times higher than reported previously for the parent estrogens. Catechol estrogens have potent endocrine effects and, because of their normal occurrence in the hypothalamic-pituitary axis, they may have an important role in neuroendocrine regulation.*

Although the catechol estrogens, including 2-hydroxyestradiol ($2OHE_2$) and 2-hydroxyestrone ($2OHE_1$), have been recognized as major urinary metabolic products of estrogen for over 20 years (*1*), their presence in tissues has never been established. The instability of catechol estrogens, as well as the relatively low concentrations of estrogens in tissues in general, has hampered previous attempts at identifying catechol estrogens in situ (*2*). Recent evidence indicates that catechol estrogens are not only metabolic end products, but possess potent biological and endocrine activities of their own (*3–6*). For this reason, we undertook a series of studies designed to identify catechol estrogens in tissues and to measure their concentrations in brain and other neuroendocrine organs.

Several years ago our laboratory described the formation of a stable radioactive *O*-methylated derivative of catechol estrogen following the incubation of estradiol with liver microsomes, reduced nicotinamide-adenine dinuncleotide phosphate, catechol-*O*-methyltransferase (COMT; E.C. 2.1.1.6) of rat liver, and [^{14}C]methyl-*S*-adenosylmethionine (*7*). Since COMT specifically *O*-methylates dihydroxylated phenols, such as the catecholamines (*8*) and catechol estrogens (*9*), it seemed feasible to utilize this enzyme to form a stable methoxy derivative for the measurement of catechol estrogens in biological materials. By employing a radioactive methyl donor of high specific activity ([^{3}H]methyl-*S*-adenosylmethionine), a partially purified preparation of COMT, and selective solvent extraction with nonpolar solvents, an extremely sensitive (< 10 pg) assay for catechol estrogens was developed (*10*). We now report the presence of catechol estrogens in various brain and endocrine tissues of the rat; and in concentrations that exceed those of their parent estrogens.

Female Sprague-Dawley rats (180 to 200 g) were obtained from Zivic-Miller and housed with free access to food and

water. Surgically ovariectomized female rats (2 weeks after operation), along with sham-operated controls, were obtained from the same source and housed under identical conditions. In some experiments, ovariectomized female rats were injected subcutaneously with 100 μg of estradiol-17β (in 0.1 ml of sesame oil) daily for 5 days. Control rats were given sesame oil alone. Rats were killed by decapitation and tissues were immediately removed and placed on ice. Blood from two to three rats was collected in heparinized tubes just after decapitation and pooled. Immediately after dissection, all tissues were frozen on Dry Ice and stored at − 20°C. Tissue specimens were thawed just prior to assay and homogenized in 25 times their volume of 0.1*N* HCl. The homogenate was extracted with three volumes of anhydrous ethyl ether (Mallinckrodt), then centrifuged at 10,000*g* for 10 minutes. The extraction procedure was repeated three times and the supernatants from the first two extracts were pooled and evaporated under a stream of nitrogen. The third extract was treated similarly and used for estimating the extraction efficiency. Plasma samples (0.25 to 2 ml) were extracted twice with six volumes of ethyl ether. After evaporation, the sediment was resuspended in 200 μl of buffer (0.1*M* tris-HCl, *p*H 7.6). Standard curves were constructed by using various concentrations of authentic $2OHE_1$ and $2OHE_2$. The authentic standards were assayed simultaneously with the tissue samples and, after being corrected for the extraction efficiency, they were used in measuring the concentration of these catechols in the tissue or plasma samples. The *O*-methylation reaction was initiated by the addition of 50 μl of a solution containing the following: 25 μl of 0.1*M* tris-HCl buffer, *p*H 7.6; 10 μl of 1*M* $MgCl_2$; 10 μl of partially purified COMT (*11*); 5 μl (2.5 μc) of [^{3}H]methyl-*S*-adenosylmethionine with a specific activity of 11.2 c/mmole (New England Nuclear). Blanks consisted of heated enzyme including substrate (that is, nonradioactive standards, tissue, or plasma extracts) or buffer with no substrate. After incubation for 60 minutes at 37°C the reaction was terminated by the addition of 0.5 ml of 0.5*M* borate buffer, *p*H 10. The radioactive *O*-methylated catechol estrogens were extracted into 6 ml of *n*-heptane (Fisher Scientific) by shaking for 30 seconds; the organic phase was then separated by centrifugation. This extraction procedure has been shown to result in little or no extraction of radioactive *O*-methylated catecholamines (*10, 12*) and also results in low blank values. Duplicate 2-ml portions of the organic phase were placed in scintillation counting vials and evaporated to dryness at 80°C in a chromatography oven. To determine "total catechol estrogens," 10 ml of Aquasol was added to one set of vials and the radioactivity was counted. To measure and identify the individual radioactive methoxy catechol estrogens, nonradioactive 2-hydroxyestradiol-2-methyl ether ($2ME_2$), 2-hydroxyestradiol-3-methyl ether ($3ME_2$), 2-hydroxyestrone-2-methyl ether ($2ME_1$), and 2-hydroxyestrone-3-methyl ether ($3ME_1$) (10 μg of each in 1 ml of ethanol) were added to the other set of vials. The volume of these samples was reduced under a stream of nitrogen and then subjected to unidimensional and bidimensional thin-layer chromatography in several solvent systems (*13*). The nonradioactive standards were visualized by briefly exposing the plates to iodine vapor.

Table 1. Concentrations of catechol estrogens in various brain and endocrine tissues of the female rat. Results are expressed as the mean ± standard error; *N* indicates the number of animals analyzed for each group.

Tissue	Total catechol estrogens (ng/g)	*N*
Pituitary	31.21 ± 2.64	4
Hypothalamus	15.53 ± 1.20	4
Cerebral cortex	9.40 ± 1.06	4
Liver	5.68 ± 0.54	8
Ovary	3.79 ± 0.41	4
Plasma	0.38 ± 0.02*	6

*The mean ± standard error of six separate determinations on pooled plasma from two to three rats. Data are expressed as nanograms per milliliter.

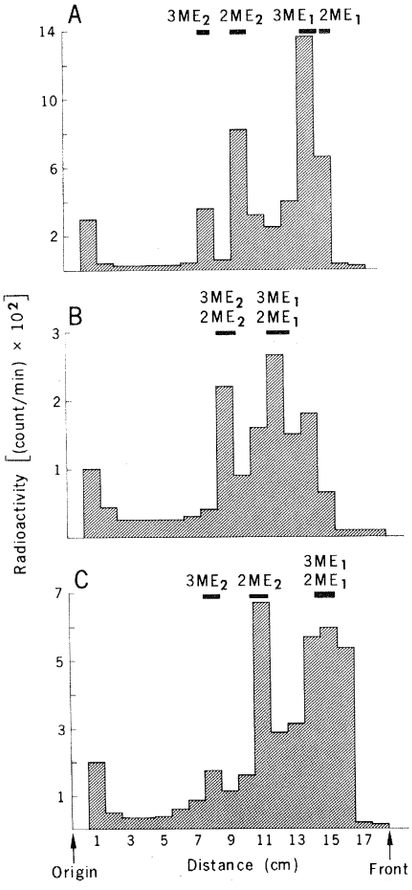

Fig. 1. Thin-layer chromatography of the ^{3}H-labeled methoxy estrogens formed from the endogenous catechol estrogens of rat hypothalamus. The solvent systems used [(i) in A, (ii) in B, (iii) in C (*13*)] allowed good separation of the reaction products of 2-hydroxyestradiol and 2-hydroxyestrone. Nonradioactive standards were visualized by exposure to iodine vapor (see text for details).

Greater than 90 percent of the radioactivity cochromatographed with the four nonradioactive standards (Fig. 1). In our initial experiments the identity of the reaction products, 2-methoxyestrone and 2-hydroxy-estrone-3-methyl ether was confirmed by subjecting the products (after first separating them by thin-layer chromatography) to mass spectral analysis (*14*). The identification of the methoxy derivatives by several chromatographic solvent systems, the absolute specificity of the COMT reaction for catechols, and the mass spectral analysis of the reaction products clearly establishes the presence of these compounds in tissues. The concentrations of catechol estrogens (*15*) are higher in the hypothalamus and pituitary than in the liver (Table 1). Since only free (nonconjugated) catechol estrogens are capable of being *O*-methylated, the lower values in liver might be explained by this organ's well-known capacity for conjugation of steroids or the brain's capacity for deconjugating such steroids (*16*).

Animals treated with estradiol-17β have markedly increased concentrations of catechol estrogens in the liver (5.68 ± 0.54 ng/g compared to 31.4 ± 2.16 ng/g, $P < .005$); the concentrations are also increased, but to a lesser extent, in other tissues. Ovariectomy alone, while decreasing the catechol estrogen concentration of plasma and liver, had less effect on brain concentrations at 2 weeks after ovariectomy. Plasma concentrations of total catechol estrogens were not markedly different between groups of intact females when pooled blood was used; however, treatment with estradiol increased plasma catechol estrogen levels significantly.

There have been only a few reports concerning the endogenous levels of estrogens in neuroendocrine tissues. In the rat, Challis and co-workers (*17*) reported estrogen levels of approximately 1 ng/g

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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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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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