Regulation of Carcinogen Metabolism in the Rat Ovary by the Estrous Cycle and Gonadotropin

Abstract. *The activity of 7,12-dimethylbenz*[a]*anthracene hydroxylase in the rat ovary is several times higher in the proestrous phase of the estrous cycle than in the estrous and metestrous plus diestrous phases. Administration of gonadotropin leads to a similar increase in the capacity of the ovary to metabolize xenobiotics. This variation in the activity of 7,12-dimethylbenz*[a]*anthracene hydroxylase during the estrous cycle may be related to the marked changes in the incidence of ovarian cancer during menopause and in women taking contraceptive pills.*

A large proportion of all cancers is believed to have a chemical etiology (*1, 2*). Ovarian cancer is one of the common types of cancer in women (*3, 4*), and its incidence appears to increase during menopause (*3, 4*). Indeed, induction of ovarian tumors in mice by carcinogenic polycyclic aromatic hydrocarbons (PAH's) such as 7,12-dimethylbenz[*a*]-anthracene (DMBA) and benz[*a*]pyrene may be preceded by a destruction of primordial oocytes (*5*). A similar mechanism may explain the high correlation of early menopause with ovarian cancer in humans living in polluted urban regions (*6*).

The toxic and carcinogenic effects of PAH's are assumed to involve activation of these hydrocarbons through microsomal cytochrome P-450–dependent hydroxylase systems to reactive epoxide intermediates (*7–9*). Cell destruction or transformation of normal cells to cancer cells may be consequences of covalent binding of a fraction of these intermediates to protein and DNA; however, the bulk of the expoxides are inactivated by epoxide hydrolase (EH) and conjugating enzymes such as glutathione *S*-transferase (GST) (*7–9*). During the metabolism of PAH's reactive quinone intermediates are also formed, which may be reduced to inactive dihydroquinones by DT-diaphorase (DT) [NAD(P)H; E.C. 1.6.99.2] (*10*).

Since the primary step in the activation of PAH's involves cytochrome P-450–dependent hydroxylases, these are of great importance in the overall process of chemical carcinogenesis. In rat liver (*11*), adrenal gland (*12*), and testis (*13*) the activity of benz[*a*]pyrene hydroxylase is influenced by large changes in the levels of pituitary hormones, such as growth hormone, adrenocorticotropic hormone, and luteinizing hormone. Although pituitary regulation of benz[*a*]pyrene hydroxylase in these organs has not been demonstrated under physiological conditions, PAH-metabolizing hydroxylases in endocrine organs may have a role in steroid metabolism.

Mouse and rat ovaries have been shown to contain benz[*a*]pyrene hydroxylase (*14*), the activity of which appears to be related to the capacity of PAH's to generate ovarian cancer (*15*) and destroy oocytes (*16*). Since the functions of the ovary are strictly controlled by the pituitary during the different phases of the estrous cycle, the ovary appears ideal for studies of the possible physiological role of the pituitary in the regulation of the activities of PAH-metabolizing hydroxylases.

We measured microsomal and mitochondrial cytochrome P-450, microsomal DMBA hydroxylase and EH, and soluble DT and GST in the rat ovary during the proestrous, estrous, and metestrous plus diestrous phases of the estrous cycle. The same activities were also measured after treatment of rats with pregnant mare serum gonadotropin (PMSG). The activity of DMBA hydroxylase during proestrous increased to 3.5 pmole/min per milligram of protein, a level about four times higher than that measured during estrous and metestrous plus diestrous (Table 1). [The metestrous and diestrous phases were not investigated separately since each has approximately the same hormonal dependence (*17, 18*).] Changes in the level of microsomal cytochrome P-450 paralleled the changes in DMBA hydroxylase activity. The activities of EH, DT, and GST were essentially independent of the estrous cycle.

The administration of gonadotropins causes pituitary control of the ovary to be bypassed and stops the normal estrous cycle in the proestrous or estrous phase (*19*). Gonadotropins thus provide a means of determining whether the estrous cycle dependence of DMBA hydroxylase is due to pituitary control. We found that the activity of DMBA hydroxylase was about three times higher in rats given PMSG than in control rats (Table 1). Administration of PMSG also led to increased levels of microsomal and mitochondrial cytochrome P-450, whereas the activities of EH, DT, and GST were essentially unchanged. Preliminary data indicate that ovarian benz[*a*]pyrene hy-

Table 1. Effect of estrous cycle and gonadotropin on ovarian detoxification activities. Female Sprague-Dawley rats weighing 250 g were maintained under a 12-hour light-dark cycle. Vaginal smears were obtained daily. Only animals that showed two consecutive 4-day estrous cycles were used in the determination of estrous cycle–dependent activities. The rats were divided into proestrous, estrous and metestrous plus diestrous groups. Each group contained at least five rats. Rats to be treated with gonadotropin showed a random estrous-cycle distribution. Gonadotropin (75 I.U. of PMSG) was administered in 0.5 ml of 0.9 percent NaCl to five rats for two consecutive days; five control rats received only 0.5 ml of 0.9 percent NaCl. After the animals were decapitated their ovaries were immediately removed, pooled, and fractionated; subcellular fractionation and preparation of microsomes and mitochondria were carried out as described by Montelius *et al.* (*24*). Protein was determined by the Lowry method (*25*). DMBA hydroxylase activity was assayed with [^{14}C]DMBA as the substrate (*24*), microsomal EH with tritiated styrene-7,8-oxide (*26*), soluble GST with 1-chloro-2,4-dinitrobenzene (*27*), and soluble DT with menadione coupled to reduced cytochrome *c* (*28*). The content of mitochondrial and microsomal cytochrome P-450 was measured from the sodium dithionite–reduced minus oxidized spectrum in the presence of carbon monoxide; both cuvettes had been reduced with phenazine ethosulfate and ascorbate (*29*). Values are means ± standard errors. Numbers of determinations are given in parentheses.

Substance evaluated	Phase of estrous cycle			PMSG
	Proestrous	Estrous	Metestrous plus diestrous	
Microsomal cytochrome P-450 (nmole/mg)	0.045 (1)	0.009 (1)	0.019 (1)	0.057 (1)
Mitochondrial cytochrome P-450 (nmole/mg)	0.099 (1)	0.071 ± 0.01 (3)	0.119 ± 0.02 (3)	0.176 (1)
DMBA hydroxylase (pmole/min-mg)	3.50 ± 1.18 (3)*	0.67 ± 0.45 (3)	1.06 ± 0.56 (3)	9.37 ± 2.63 (3)†
EH (nmole/min-mg)	0.823 ± 0.03 (2)	0.558 ± 0.05 (2)	0.687 ± 0.05 (2)	0.695 ± 0.08 (2)
DT (nmole/min-mg)	105.5 (1)	122.0 ± 52.9 (4)	92.73 ± 33.1 (4)	105.1 (1)
GST (nmole/min-mg)	362.3 ± 34.6 (2)	374.4 ± 51.2 (4)	349.1 ± 50.2 (4)	318.0 (1)

*Significantly different from the activity in the estrous phase ($P < .05$, Student's *t*-test). †Significantly different ($P < .01$) from the control activity (2.90 ± 0.23 pmole/min per milligram of protein; three determinations).

droxylase is regulated by the estrous cycle and gonadotropins in a manner similar to that of DMBA hydroxylase.

Our results indicate that ovarian DMBA hydroxylase is regulated by the pituitary and that EH, DT, and GST are not. To our knowledge, this is the first time that DMBA hydroxylase has been shown to undergo short-term cyclic changes controlled by endogenous factors under physiological conditions. This finding may cast light on the role of PAH-metabolizing hydroxylases in endocrine organs. The estrous cycle dependence of DMBA hydroxylase suggests that the normal substrate is a steroid, a possibility supported by the sensitivity of this enzyme to 17-hydroxylase inhibitors (*20*). Physiologically, the relation between the level of gonadotropins secreted by the pituitary and DMBA hydroxylase activity indicates that the rate of ovarian cancer initiation increases under conditions of increased secretion of gonadotropins. This is supported by the finding that excessive stimulation of grafted rat ovaries by gonadotropins, in the presence or the absence of added xenobiotics, leads to an increase in ovarian cancer (*21, 22*). Thus the action of gonadotropins on DMBA hydroxylase may be one of several hormone-dependent effects, including those at the level of gene expression and DNA replication. It is interesting to note the high incidence of ovarian cancer following menopause (*3, 4*) and the low incidence in women taking contraceptive pills (*23*), conditions that lead to increased and decreased levels of gonadotropins, respectively.

Ovarian benz[*a*]pyrene hydroxylase is induced by PAH's (*14*). The relation between the cellular mechanism in pituitary hormone regulation and PAH regulation of DMBA hydroxylase and benz[*a*]pyrene hydroxylase remains to be elucidated, as does the clinical significance of the pituitary regulation of PAH-metabolizing hydroxylases.

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Tension Transients in Single Isolated Smooth Muscle Cells

Abstract. *Tension transients were recorded in a single smooth muscle cell. The transient contains a linear elastic response and a biphasic recovery that appear to originate from the cross-bridges. A comparison of transients in smooth and fast skeletal muscle fibers suggests that the cross-bridge in smooth muscle is more compliant than the cross-bridge in striated muscle and that transitions between several cross-bridge states occur more slowly in smooth muscle than in striated muscle.*

The cyclic interaction of myosin cross-bridges with actin filaments in smooth muscle is believed to be responsible for force generation or active cell shortening (*1*). Although the contractile mechanism in smooth muscle is similar to that in striated muscle, the contraction of smooth muscle has several distinctive features. The velocity of shortening and rate of the actomyosin adenosine triphosphatase are much slower in smooth muscle than in striated muscle. In addition, maximum force production in smooth muscle is comparable to that in striated muscle but requires much less myosin. These differences may be explained in part by differences in the cross-bridge cycle. As several steps of the cycle are believed to be sensitive to the position of the cross-bridge relative to its actin binding site, rates of transition between cross-bridge states (that is, cross-bridge kinetics) may be tested by application of small force or small length perturbations (*2*). The multiphasic tension transients observed in frog skeletal muscle in response to small and rapid changes in length are believed to reflect the kinetics of various steps in the cross-bridge cycle. Similar studies have been performed on several smooth muscles (*3, 4*), but marked differences in the tension transients were observed in different tissues. Furthermore, none of the tension transients observed in smooth muscle were similar in form to those observed in single striated muscle fibers. Although the differences in the tension transients of smooth and striated muscle might reflect real differences in their contractile machinery, they might also reflect differences in preparation—for example, a single striated muscle fiber compared with a more complex multicellular segment of smooth muscle.

We investigated the force-generating mechanism in smooth muscle by observing the response of single isolated smooth muscle cells (SMC) to rapid changes in length during isometric force production. For this purpose, we developed the means to measure force from such a small cell, as well as the techniques required to attach the cell to a force-measuring device (*5*). Single SMC were obtained by enzymatic disaggregation of the stomach muscularis of the toad *Bufo marinus*. These cells are typically 150 μm long and 6 μm wide and have no tendinous connections. A short portion of each end of a single cell was tied by micromanipulation around special probes. The probes were connected to a length driver for precise control of cell length and to a newly designed force transducer for measurement of the force response. The new transducer, which has greater resolution (1 μg) and a higher natural frequency (425 Hz) than those used previously for recording isometric