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# Increased Gastrointestinal Motility *in Vitro* following Chronic Estrogen Treatment in Male Rats (41073)[1]

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*Abstract.* The influence of 17β-estradiol ($E_2$) on contractile activity of three gastrointestinal regional tissues was evaluated. One of three dose levels (75, 300, and 600 mg/kg/day) of $E_2$ was administered sc to groups of male rats daily for 4 days. $E_2$ blood concentrations measured with radioimmunoassay on the fourth day were 3.9, 28.9, and 51.4 ng/ml for the respective treatment groups. Significant differences were demonstrated in contractile activity responses between tissues from $E_2$-exposed and nontreated control animals when the tissues were challenged with a cholinergic agonist *in vitro*. Evaluation of the data with regression analysis suggests differences in sensitivity among the three regional tissue responses when correlated with serum $E_2$ concentrations; i.e., colonic responses > esophageal responses > antral responses. A causal relationship between elevated serum estrogen concentration and gastrointestinal hyperactivity is suggested.

Estrogens play a major role in reproductive physiology. Combinations of exogenous estrogens and progestins are taken for birth control. Estrogens are administered for postmenopausal problems and for postmyocardial infarction therapy. Unwanted side effects of estrogenic administration may include gallbladder problems, hyperlipidemia, hypertension, and blood clotting deficiencies. Furthermore, estrogen is cited as a potential carcinogenic agent (1–6). However, the benefits of estrogen therapy are obvious.

"Morning sickness," gastroesophageal reflux (heartburn), and colonic disturbances may result from abnormal regional gastrointestinal contractile activity when serum hormonal levels are high. Most clinical trials and investigations in this area have used combined estrogen and progesterone regimes (7–9). It is difficult to assess the influence of estrogen or progesterone alone on a given system based on current information because of the reported action of the estrogen potential to increase progesterone receptors in uterine tissue (10). We have previously reported a progesteronic inhibitory influence on gastrointestinal motility induced by chronic progesterone exposure (11–13). Establishment of an estrogenic influence alone on gastrointestinal tissue is necessary in order to evaluate the secondary effects of the estrogen component in oral contraceptives and estrogen therapy in pre- and postmenopausal women.

We report here an excitatory estrogenic influence on gastrointestinal motility. The responses were present at serum estrogen levels which are comparable to ranges occurring in women during pregnancy or receiving oral contraceptives.

Tissues from the esophagus, antrum, and colon were tested. These three regions were chosen because of the different functional role each plays in the normal digestive process and because recipients of oral contraceptives (7), gravid women (8, 9, 14) and premenopausal women (15) can experience problems in each of these separate regions.

**Materials and Methods.** The male rat was used in this study to avoid cyclic fluctuations of estrogen levels occurring in the female. Groups of male Sprague–Dawley rats (190–210 g) were administered 17β-estradiol in three doses (75, 300, 600 μg/kg/day) in sesame seed oil (0.1 ml) sc for 4 days at 24-hr intervals. Paired control animals received the vehicle only. On the

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fourth treatment day (2 hr after final injection) blood samples were taken for determination of serum estradiol by radioimmunoassay (16) and sections of three regional tissues, esophagus, antrum, and colon, were excised from paired control and 17β-estradiol-treated animals and prepared for assessment of motility parameters *in vitro* as previously described (11, 12).

*Tissue isolation.* The excised tissue from the respective gut regions were maintained under optimal *in vitro* conditions throughout the experiment (Krebs–Ringer solution (11), pH 7.4; 37 ± 0.5°; 5% $CO_2$–95% $O_2$). Circular rings of tissue taken from the animals were incised and both ends ligated with suture and placed in 30-ml baths. One end was attached to a stabilization bar and the free end connected to a tension tranducer (Grass model Ft. 0.03-force displacement transducer). Thus, the tissue responses reflect circular muscle activity. A 30-min equilibration period in the muscle baths was allowed for each preparation. Optimal basal tension levels ($T_0$) for each type of regional tissue were obtained as previously described (13) and placed on the tissues at the beginning of the equilibration period and maintained at 5-min intervals throughout the course of the experiment. Spontaneous tissue activity was quantitated for the first 5-min (control) period following the equilibration period. Chemical stimulation of cholinergic receptors in the tissue preparations was achieved with a single dose of arecoline–HCl ($1 \times 10^{-6}$ *M*, bath conc.) administered into the muscle baths with micropipets (vol 50 μl) after the initial 5-min control period. This arecoline concentration represents an approximate $ED_{70}$ for the three regional tissues (12). Tissue responses were then quantitated for each preparation for three sequential 5-min intervals. Each tissue response represents 15 min of tissue activity divided by 3 (three 5-min recording periods) minus the tissue's spontaneous activity in the control period. The dissimilarity among regional tissue responses necessitated quantitation of the data in three different formats. Chemically challenged esophageal tissue responded with a single tonic contraction and was quantitated as force of contraction per minute. Antral tissue responses were phasic and these responses were calculated as force of contraction per contraction. Phasic and tonic contractions were recorded from colonic tissue and this activity was quantitated as a motility index which equalled frequency × force of contraction (12). Differences between responses of tissues from a treated and a paired nontreated (vehicle only) animal are expressed as a percentage of the nontreated (control) response. No less than 10 animal pairs were used for each 17β-estradiol dose level. The percentage difference in the tissue responses between each paired group constitutes the data base from which each dose level activity mean and the respective regression analyses were derived.

*Data quantitation.* A detailed description of the recording system may be found elsewhere (13, 17). Briefly, the transducer signal was transmitted simultaneously to an integrator and a fiber optic-cathode ray

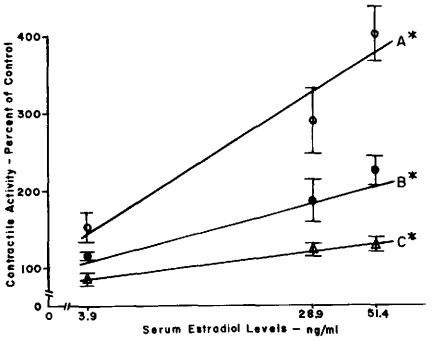


FIG. 1. The effects of chronic estrogen exposure *in vitro* on the contractile activity of colonic (○), esophageal (●), and antral (△) tissue *in vitro*. The regression lines were derived from the percentage difference between the tissue responses from each animal pair (control vs treated) plotted against the log serum estradiol levels. The data points represent the respective tissue response group means ± SEM ($N$ = 10). The regression lines are described as: (A)*, colonic tissue: $Y = 27.3 + 199.3 \log x$ ($r = 0.56$); (B)*, esophageal tissue: $Y = 58.4 + 85.5 \log x$ ($r = 0.64$); and (C)* antral tissue: $Y = 60.3 + 40.9 \log x$ ($r = 0.65$) where $\log x$ = log serum estradiol level. (Each of the data points at each serum level represents a comparison between the respective tissue responses from 10 treated and 10 control animals.)

tube recorder (Honeywell). Contractile activity was integrated in terms of g·sec to give the total force of contraction (units: g·sec) for the average of three 5-min recording periods. Frequency of contraction for each 5-min period was also measured.

*Serum 17β-estradiol extraction and assay.* One milliliter of blood was collected by cardiac puncture from each animal concurrently with the excision of gut tissue. The blood samples were carefully taken 2 hr after the final 17β-estradiol or vehicle injection.

Serum samples (0.5 ml, in duplicate) were extracted twice with 3 ml of ethyl ether, ether dried, and extract was stored at −20° until assayed. The percentage recovery through the extraction procedure was 79.5 ± 4.6% (±SEM) as determined from the recovery of [$^3$H]estradiol (90.7 Ci/mmole, New England Nuclear) added to the incubation medium or to "pooled sera" from control male animals. 17β-Estradiol in serum was measured by radioimmunoassay (16) using an antibody to 17β-estradiol (18). Binding of [$^3$H]estradiol to the antibody was an average of 24.3% for three assays. The interassay and intrassay variations were 8.3 and 11.8%, respectively. Sensitivity of the assay was 3 pg/tube. Linearity of the standard curve ranged from 3 to 400 pg/tube.

*Statistical analysis.* Statistical evaluations of 17β-estradiol blood concentrations and the respective tissue response group means were performed by unpaired *t* test (19). Regression analysis and median effective dose ($ED_{50}$) values were calculated according to methods described by Colton (20). Values of $P < 0.05$ were considered significant.

**Results.** The three regional tissue contractile activities are plotted against serum estradiol levels and described by regression lines in Fig. 1 and the values are given in Table I. Statistical comparison of the re-

TABLE I. SPONTANEOUS, CONTROL AND ESTRADIOL TISSUE RESPONSES GENERATED BY THREE REGIONAL TISSUES

| | Esophagus g-sec/min[a] | | Antrum g-sec/contraction | | Colon g-sec × freg | |
|---|---|---|---|---|---|---|
| | C[b] | T[c] | C | T | C | T |
| Spont[d] | 9.98[e] ±1.92 | 9.41 ±2.13 | 0.025 ±0.020 | 0.017 ±0.010 | 0.012 0.01 | 0.014 0.01 |
| $E_2$-3.9[f] ng/ml | 34.90 ±4.3 | 40.4 ±4.4 | 1.92 ±0.47 | 1.55 ±0.30 | 982 ±204 ▲[g] | 1488 ±191 |
| Spont | 11.65 ±3.64 | 11.49 ±2.49 | 0.01 ±0.002 | 0.01 ±0.002 | 0.002 ±0.0013 | 0.002 0.0016 |
| $E_2$-28.9 ng/ml | 37.81 ±4.89 ▲ | 70.89 ±5.84 | 1.42 ±0.46 | 1.71 ±0.37 | 857 ±313 | 2418 ±214 ▲ |
| Spont | 9.04 ±3.07 | 10.64 ±4.03 | 0.03 ±0.02 | 0.02 ±0.01 | 0.02 ±0.01 | 0.02 ±0.02 |
| $E_2$-51.4 ng/ml | 22.79 ±3.29 ▲ | 51.39 ±5.36 | 1.44 ±0.16 ▲ | 1.85 ±0.17 | 935 ±333 ▲ | 3686 ±767 |

[a] See Materials and Methods for explanation.
[b] Control—tissues from nontreated animals.
[c] Treated—tissues from estradiol exposed animals.
[d] Spontaneous Activity for each respective tissue group.
[e] Each value in table represents mean ± SEM from the respective tissue responses from 10 animals (three regional tissues from one animal).
[f] $E_2$—estradiol serum level of animals in treated group. Both C and T tissues were challenged with arecoline 1 μ*M*.
[g] ▲, Significant difference between C and T ($P < 0.05$).

gression lines indicates that each line and slope is significantly different from the other two ($P < 0.05$). The slope of each regional tissue response curve is significantly different from the others, with colonic tissue showing the most sensitivity. The slope for the antral response curve is not significantly different from the control response curve ($P > 0.05$). Analysis of the mean values of the tissue responses indicates a significant antral response only at the highest serum estradiol level, significant esophageal responses at the two highest levels, and significant colonic responses at all three concentrations measures ($P < 0.05$). No significant differences could be detected in the frequency of contraction of the treated animal tissues compared to the nontreated animal tissues ($P > 0.05$).

The serum 17$\beta$-estradiol concentrations measured from the control animals in this study ranged from 41 to 72 pg/ml with a mean value of 53.4 pg/ml ($N = 36$). The serum 17$\beta$-estradiol concentrations measured from the treated animals are given in Table II. The relationship between the 50% tissue responses and the calculated serum 17$\beta$-estradiol concentrations at that response level are shown in Table III. The data demonstrate that colonic and esophageal tissues are influenced by chronic estradiol exposure. Antral tissue responses at the 50% response level are not significantly different from control tissue responses ($P > 0.05$).

The serum estradiol concentrations of animals treated with the lowest estradiol dose are comparable to estrogen levels found in women taking oral contraceptives (3.8 to 4.2 ng/ml) (21). The serum levels attained in the higher estradiol treatment groups are within the range of serum estrogen concentrations reported during human pregnancy (30 to 40 ng/ml) (22).

TABLE II. 17$\beta$-ESTRADIOL ADMINISTERED DOSE LEVELS, AND RESULTING SERUM CONCENTRATIONS

| Dose levels $\mu$g/kg/day (4 days) | Serum levels (ng/ml) |
|---|---|
| 75 | 3.9 ± 0.2[a] |
| 300 | 28.9 ± 1.2 |
| 600 | 51.4 ± 2.4 |
| Control (vehicle only) | 0.0534 ± 0.0012 |

[a] $\bar{x}$ ± SEM.

TABLE III. COMPARISON OF THE 50% TISSUE RESPONSE WITH THE MEDIAN EFFECTIVE 17$\beta$-ESTRADIOL SERUM CONCENTRATIONS ($ED_{50}$) FOR THE THREE REGIONAL TISSUES

| | 50% Tissue response (Percentage of control) | Serum 17$\beta$-estradiol $ED_{50}$ values (ng/ml) |
|---|---|---|
| Colon | 272 sign[a] | 16.9 |
| Esophagus | 170 sign | 20.2 |
| Antrum | 105 N.S.[b] | 12.3 |

[a] ($P < 0.05$).
[b] ($P > 0.05$).

**Discussion.** The colonic tissue response induced by chronic estradiol exposure, as demonstrated in this study, suggests an estrogenic influence on colonic activity when serum estrogen levels are high in the female. Although the magnitude of the esophageal and antral tissue response is less than that of the colonic tissue response, these regions may be affected by the higher serum estradiol levels during pregnancy.

Our results are in agreement with studies in which chronic estrogen exposure (5–20 $\mu$g/kg/day) has been reported to increase the rate of intestinal transport in rats *in situ* (23) and intestinal and stomach electrical activities in dogs *in situ* (24). However, other studies report an immediate inhibitory effect of estrogen or lower esophageal sphincteric muscle preparations *in vitro* (25). Evidence from a recent study (26) may clarify the apparent paradox observed in chronic and acute estrogen exposure studies. Comparison of spontaneous uterine contractions measured *in situ,* in ovariectomized rats before and after estrogen treatment, revealed a significant reduction in frequency of the contractions, but a significant increase in the force of contraction after a chronic 12-hr exposure. The duration of the estrogen exposure seems to make a difference. Within the protocol of the present experiment allowing for 4 days of estrogen exposure, only the excitatory effects of estrogen were detected.

Estrogen is known to promote an increase in contractile protein content and

excitability of uterine smooth muscle (27). The results of this study indicate an analogous excitatory estrogenic influence on contractile activity in certain gastrointestinal regions when serum estrogen levels are high relative to serum progesterone levels. However, evidence from this laboratory (11–13) and others (8, 9) suggests that the contractile activity of gastrointestinal tissues is decreased when serum progesterone concentrations are within the physiological levels seen in pregnancy. The predominate steroidal effect on gastrointestinal motility, when both steroids are present in the serum, remains questionable and is further complicated by the potential ability of estrogen to induce intracellular progesterone receptor formation if gastrointestinal smooth muscle responds to estrogen similarly to uterine smooth muscle.


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