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INHIBITION OF GASTRIC ACID SECRETION BY ESTRADIOL-17β IN FEEDING-CONTROLLED RATS

PAWINEE PIYACHATURAWAT and LIANGCHAI LIMLOMWONGSE

Department of Physiology, Faculty of Science, Mahidol University, Rama VI Rd., Bangkok 4, Thailand

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Abstract

Inhibitory effect of estradiol-17β on gastric acid secretion was studied in ovariectomized rats and compared with sham-operated rats. All rats were subjected to controlled feeding to equalized the food intake. Depression of the acid secretion was found in all estrogen-treated rats of both the low food intake group and the group which was forced-fed with normal amounts of food. The level of plasma glucose was significantly elevated by estrogen treatment whereas the levels of plasma calcium concentration and plasma osmolarity were unaffected. The results indicated that the inhibitory effect of estrogen on acid secretion was not primarily due to its effect on food intake. The possible mechanisms were discussed.

Introduction

Estrogen has been reported to depress acid secretion [1,2] but the mechanism whereby the estrogen acts is unknown. Several experiments have shown a relationship between neural mechanism controlling gastric secretory activities and food intake. For example, rats with ventromedial hypothalamic (VMH) lesions become gastric hyperacidic and hyperphagic [3]. This central control of food intake appears to be affected by estrogen in that systemic injections as well as intracranial implantation of crystalline estradiol into the VMH region resulted in the reduction of food intake [4,5]

In view of the possible relation between the inhibitory effect of estrogen on acid secretion and food intake, the present experiment tested whether the reduction in acid secretion produced by estrogen was a consequence of its influence on food intake. Two paired-feeding procedures were emloyed: first, the food of the control animals was restricted to the level of estrogen-treated animals and second, the control and estrogen-treated animals were force-fed with equal amounts of food.

Methods and Materials

Female rats (Fischer strain) weighing 140–150 g were housed in individually in wire cages. The experimental room was illuminated for 12 h each day and maintained at a temperature of 25°C. The rats were divided into two groups: one group served as sham-operated control and the other was ovariectomized and treated with estrogen. Five days after ovariectomy, the animals were injected intramuscularly once daily with estradiol benzoate (80 μg/100 g rat, Schering Co., Bangkok Thailand) diluted with corn oil.

Experiment 1: Pair-feeding study. In this experiment, the control rats were pair-fed with the same amount of food taken by the estrogen-treated rats. All animals were fed with standard laboratory chow (Gold Coin Ltd., Singapore). During the experimental period, the estrogen-treated rats were given free access to food at all times and the daily consumption was recorded. The same amount of food intake of estrogen-treated rats was subsequently given to the sham-operated controls and the ovariectomized ones which were injected with oil vehicle only. Food is normally consumed completely by the pair fed groups. The body weight was recorded once daily at 1800.

Experiment 2: Force-feeding study. The animals were force-fed throughout the experiment with semiliquid diet by stomach tube. Each rat received 3 g purified diet[6] mixed with 2 ml water three times daily. Feeding times were 0800, 1200 and 1800. This intake approximated the mean daily intake of animals of the same weight and age fed *ad libitum* (baseline intake). Body weight measurements were made daily for all rats prior to the evening feeding.

After 7 days of estrogen injection, all animals were analyzed for gastric acid secretory capacity by a method which was previously described[7]. Briefly, the stomach of a rat anesthesized with ether was exposed and ligated at the pyroduodenal junction. The content of the stomach was removed and the stomach was washed with physiological saline by means of a polyethylene tube passed orally. The esophagus at the neck region was ligated. The gastric acid secretion was then stimulated by an intramuscular injection of pentagastrin (Calbiochem. Co., U.S.A.) 130 μg/100 gm rat. One hour later, the rat was anaesthetized again. The sotmach was ligated at a point of entry of the esophagus and then removed along with its secretion. The differences between the full and empty stomach were noted as the weight of total gastric content. The total acid output was determined by titration with standard NaOH to pH 5 by using the Beckman automatic titrator and expressed as μEqH^+/g stomach/hr. The plasma from pair-fed rats was collected for analysis of glucose, calcium and osmolarity. The plasma glucose were measured by the glucose-oxidase and peroxidase kit No. 510 from Sigma Chemical Co., St. Louis, Mo. The osmolarity was determined by cryoscopy, and the plasma calcium was measured by the method of Pybus[8].

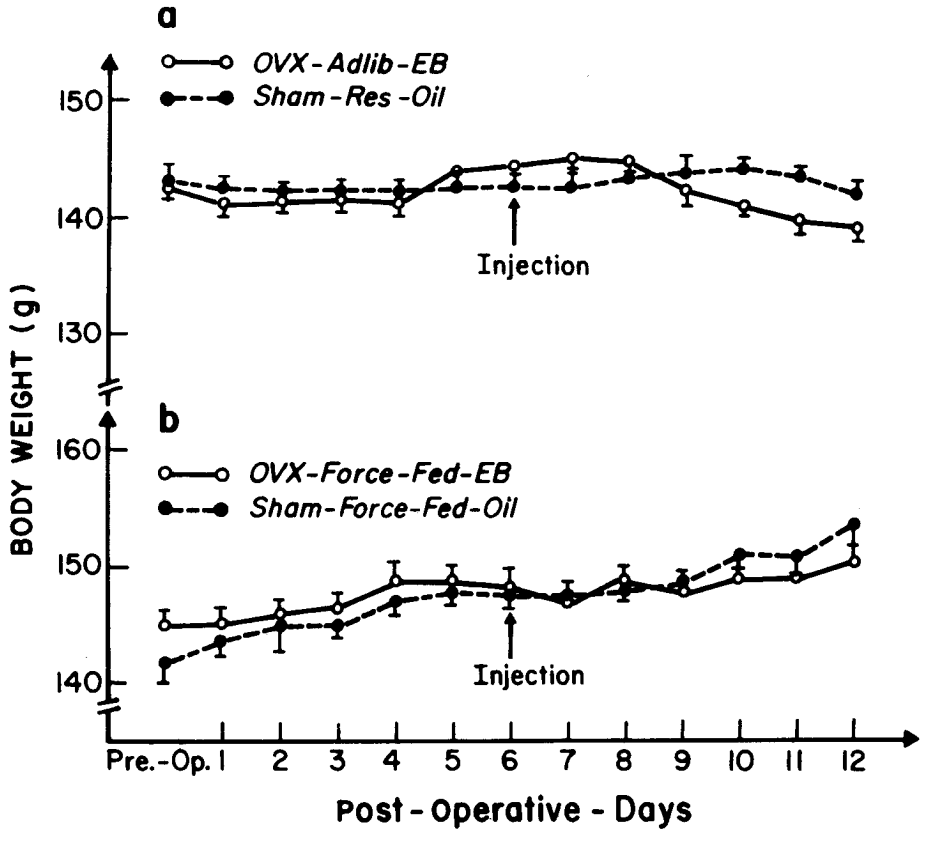

Figure 1. Effect of estradiol-17 β treatment on the body weight (g, mean ± S.E) of the rats subjected to controlled feeding.

OVX is ovariectomized rats and EB is estradiol benzoate. ***Ad lib*** **rats have** free access to an excess of food at all times and restricted rats (Res) were given a daily ration equivalent to the estrogen treated rats intake (n = 8).
Forced fed rats were fed daily ration equivalent to their base-line intakes (n = 12).

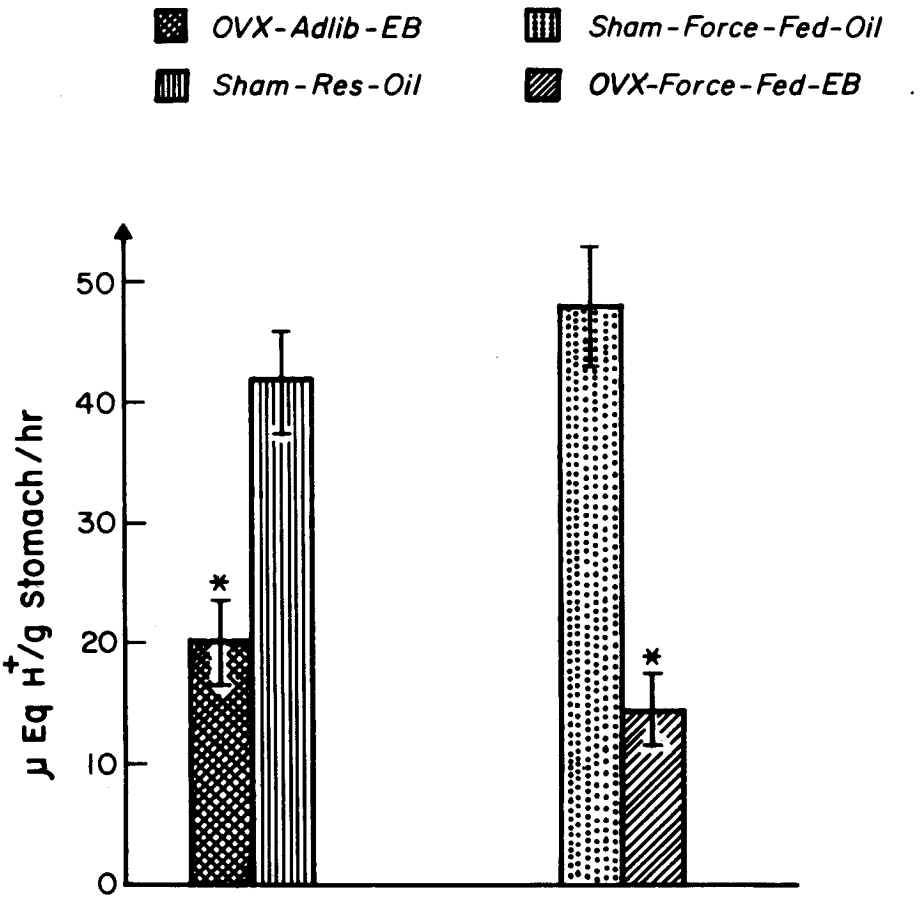

Figure 2. Rate of gastric acid secretion (μEqH^+/g stomach/hr, mean ± S.E) in response to intramuscular injection of pentagastrin 130 μg/100 g in sham-operated control and ovariectomized rats treated with 80 μg estradiol benzoate/100 g body weight.

* highly significantly different ($P < 0.001$) from the sham-operated control using Student's t-test. Abbreviations are the same as Fig. 1.

Results

An average 5% reduction in food intake of estrogen rat was observed in the experiment I after the third injection. The body weight was slightly reduced from 145 ± 2.0 g on the first day of estradiol injection to 139.2 ± 1.5 g on the seventh day of injection. When the same amount of food was pair-fed to the non-estrogen treated group, the body weight showed a similar reduction. The weights were reduced from 143.5 ± 1.4 to 141.8 ± 1.6 g in the sham control as shown in Fig. la. Unlike the pair-fed rats, the body weights of all force-fed rats were found to be increased. They were changed from 147.6 ± 0.9 to 153.9 ± 1.2 g in the sham control and from 148.7 ± 1.4 g to 151.9 ± 1.3 g in the ovariectomized rats treated with estrogen respectively. The pattern of changes in body weights of the rats with different treatments in each controlled-feeding experiment were found to be similar and their body weights were not significant different ($P > 0.05$), as shown in Fig. 1b.

Analysis of acid secretory capacity in response to exogenous gastrin showed that the acid secretion of the 7 day estrogen treated rats in both pair-fed and force-fed studies was markedly depressed and significantly different ($P < 0.001$) from the sham-operated control (Fig. 2). It was reduced from 41.9 ± 4.1 to 20.1 ± 3.6 μEqH^+/g stomach/ h in pair-fed rats and from 48.4 ± 5.0 to 14.4 ± 2.8 μEqH^+/g stomach/ h in force-fed rats. Table 1 shows the plasma levels of glucose, osmolarity and calcium concentration obtained from sham-operated and ovariectomized rats treated with estrogen. The estrogen-treated rat shows a significant higher plasma glucose level when compared to the sham ($P < 0.01$) whereas the plasma osmoarity and calcium concentration were not affected by estrogen treatment ($P > 0.05$).

Discussion

An inhibitory region for the neural control of gastric acid secretion has been demonstrated to be in the ventromedial nucleus of the hypothalamus (VMH) or satiety center[3]. For example, an increase in blood glucose[9] as well as distention of the stomach[10] resulted in an increase in the electrical activity of the nucleus. Estradiol has been reported to stimulated VMH, resulting in the reuction of the food consumption and body weight[4,5]. This was confirmed by the present study in which animals were fed *ad libitum*. With the controlled feeding methods used, the food intake of the sham-operated control and the ovariectomized rats with estrogen treatment was regulated to equal amounts and comparable weight changes in each experiment were observed. However, the marked depression in the gastrin-stimulated gastric acid secretion of estrogen-treated rat was observed in the controlled feeding experiments when compared to controls. Ovariectomy which was reported to increase the gastrin levels[11] did not significantly alter the gastric acid secretion in our study[2]. The depression of acid secretion by estrogen is unlikely to be caused by the lowering of the gastrin level. This discrepancy may be due to the difference in the species of animals. In addition, the reduction of acid secretion should not be due to the depressing effect of estrogen upon the food intake.

TABLE 1. EFFECT OF ESTRADIOL TREATMENT ON THE PLASMA GLUCOSE; OSMOLARITY AND CALCIUM CONCENTRATION IN THE PAIR-FED RATS.

	No. of rats	Glucose (mg%)	Calcium (mg%)	Osmolarity (mOsm)
Sham + Oil	12	119.8 ± 7.5	9.0 ± 0.3	298.6 ± 2.8
OVX + EB	12	150.8 ± 0.01*	9.0 ± 0.1	304.3 ± 2.8

* Highly significantly different from sham-operated controls ($P < 0.01$).

The identification of the mechanism underlying the inhibitory effect of estrogen on acid secretion is complex. Though the depressing effect of food consumption and the inhibitory impulse on acid secretion might have arisen from the same location, there is no conclusive evidence of the quantitative relation of these parameters. Moreover, there exists the interaction of estrogen with other metabolically significant systems which can participate in the alteration of gastric secretion. For example, estrogen is reported to influence the circulating levels of insulin, growth hormone, glucose, calcium and other minerals[12]. The plasma level of calcium and plasma osmolarity were also determined in this study and found to be unaltered by estrogen whereas the plasma level of glucose in estrogen treated group was significantly increased ($P < 0.01$). A reciprocal relationship between blood glucose levels and gastric acid output was previously described[13]. The inhibitory effect of glucose could be partly peripheral due to elevated plasma osmolarity, to a reduction in plasma gastrin, and partly central by way of the vagal center. However, an increase in plasma glucose of the estrogen-treated rat was occured without a change in plasma osmolarity, similar to a previous study[13]. In addition, a large amount of exogenous gastrin could not raise the acid secretory response of estrogen-treated animals to the normal level. These two peripheral actions could thus be excluded.

The high level of glucose could led to an increase in the spike frequency of satiety center neurons with a decrease in the spike frequency of feeding center neurons and the vagal activity to the stomach[14]. The increased inhibition to the vagal center by the elevation in the plasma glucose level by estrogen might serve an important role in the reduction of the secretory activity of the gastric cells. However, in relation to the hyporesponsiveness to a large amount of exogenous gastrin, the inhibitory action of estrogen at the gastric tissue level could be a possible contributory factor.

It becomes evident from this study that the inhibitory effect of estrogen on acid secretion was not primarily due to its effect on food intake. It may interfere with metabolism of other system as well as acting directly on the gastric tissue.

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บทคัดย่อ

การวิจัยนี้เพื่อศึกษากลไกของอีสตราไดออล (Estradiol-17β) ในการยับยั้งการหลั่งของกรดในกระเพาะอาหารของหนูพุกขาว (rat) โดยทำการเปรียบเทียบอัตราการหลั่งของกรดในกระเพาะอาหารที่ถูกตัดรังไข่ออกแล้วและได้รับฮอร์โมนอีสตราไดออลเป็นเวลา 7 วันกับหนูปกติ ทั้งนี้ ปริมาณอาหารที่สัตว์ทดลองกินในแต่ละกลุ่ม จะถูกควบคุมให้เท่ากันด้วยวิธีการ 2 แบบ กล่าวคือแบบแรกให้หนูปกติกินอาหารเท่ากับหนูที่ได้รับฮอร์โมนอีสตราไดออลซึ่งจะต่ำกว่าระดับการกินปกติ แบบหลังทำการป้อนอาหารแก่หนูที่ได้รับฮอร์โมนอีสตราไดออลให้เท่ากับหนูปกติ พบว่าหนูที่ได้รับฮอร์โมนอีสตราไดออลในการทดลองทั้ง 2 แบบมีอัตราการหลั่งของกรดในกระเพาะต่ำกว่าหนูปกติมาก ($P < 0.001$) ทั้ง ๆ ที่ได้รับอาหารในปริมาณที่เท่ากันและมีการเปลี่ยนแปลงของน้ำหนักตัวพอ ๆ กัน นอกจากนี้ยังพบว่าในหนูที่ได้รับฮอร์โมนอีสตราไดออลมีระดับน้ำตาลกลูโคสในเลือดสูงกว่าหนูปกติ แต่ว่าระดับของแคลเซี่ยมและความเข้มข้นของน้ำเลือดไม่แตกต่างจากหนูปกติ ผลการทดลองนี้ชี้ให้เห็นว่ากลไกของอีสตราไดออลในการยับยั้งการหลั่งของกรดไม่เกี่ยวข้องกับฤทธิ์ในการกดการกินอาหาร และได้อภิปรายถึงกลไกบางอย่างของอีสตราไดออลที่อาจจะมีผลยับยั้งการหลั่งของกรด