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DUAL EFFECT OF FEMALE SEX STEROIDS ON DRUG-INDUCED GASTRODUODENAL ULCERS IN THE RAT

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Summary

Since the sexual dimorphism of gastroduodenal ulcers is well known and might possibly relate to the actions of sex hormones, we studied the role of the female sex steroids, progesterone and 17β-estradiol in cysteamine-induced mucosal ulcers in female Wistar rats (200-220 g). Administration of cysteamine (400 mg/kg, s.c.) provoked macroscopic gastroduodenal mucosa injury as assessed planimetrically, an increase in microvascular permeability in the stomach and the duodenum as assessed by extravasation of radiolabelled albumin, and decreased gastroduodenal mucus levels as assessed by the Alcian blue technique. Ovariectomy (2 weeks before cysteamine) decreased plasma 17β-estradiol level as assessed by radioimmunoassay, gastroduodenal macroscopic injury and albumin extravasation, and increased mucus levels following cysteamine challenge. Administration of progesterone (10-50 mg/kg/week, s.c.) attenuated in a dose-dependent manner cysteamine-induced gastroduodenal mucosa injury and microvascular leakage, while it increased mucus levels in the stomach and the duodenum. In contrast, administration of 17β-estradiol (1-5 mg/kg/week, s.c.) dose-dependently augmented gastric and duodenal macroscopic mucosa lesions and microvascular injury provoked by cysteamine, and caused a further reduction in gastroduodenal mucus levels observed after cysteamine administration. In different experiments, ovariectomy decreased indomethacin-induced gastroduodenal injury. The injection of 17β-estradiol (1-5 mg/kg/week) did not affect gastroduodenal damage, while treatment with progesterone (10-50 mg/kg/week) protected against indomethacin-provoked mucosa ulcers. It is concluded that female sex steroids play a role in drug-induced gastroduodenal ulcers by modulating microvascular permeability and mucus secretion.

Key Words: female sex steroids, gastrointestinal ulcers, progesterone, 17β-estradiol

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On the basis of experimental and clinical observations, it is known that ulcers of the gastroduodenal mucosa develop in a sexually-dependent manner. For example, in the fertile age, peptic ulcer disease occurs more frequently among men than in women (1,2). This sex-dependent difference may also be found in experimental models of mucosa damage, as oral administration of ethanol generated more severe gastric erosions in male rats than in females (3,4). In this model of mucosal damage, gonadectomy protected the stomach against ethanol-induced injury only in male rats, but not in females (3). Moreover, administration of the testosterone receptor blocker, cyproterone acetate attenuated gastric hemorrhagic erosions in intact male rats following ethanol challenge. In contrast, in a different model of gastroduodenal ulcers (as induced by restraint and forced exercise), the stomach of male rats was less sensitive to mucosa damage compared to that of females (5). In conjunction to these findings, it can be suspected that sex hormones might possibly play a role in the development of gastroduodenal mucosa ulcers.

The actual sexual hormonal status of females may also be related to the generation of gastroduodenal ulcers. Pregnancy and lactation in rats markedly reduced steroid- and cysteamine-induced gastroduodenal lesions (6,7). A lower incidence of peptic ulcers is also present in pregnant women (2). Moreover, during pregnancy the frequency of hemorrhage and/or perforation from gastroduodenal ulcers is low compared to its higher incidence in puerperium (1). Thus, the elevation of endogenous progesterone plasma levels may be responsible for pregnancy-induced protection against mucosa ulcers, since early pregnant rats (with high plasma progesterone levels) were less sensitive to gastroduodenal ulcers (7,8).

The aim of the present study was to evaluate the possible role of female sexual steroids, progesterone and 17β-estradiol in the generation of gastroduodenal ulcers induced in rats by treatment with drugs such as cysteamine and indomethacin. Administration of cysteamine is known to cause gastric mucosal lesions and duodenal ulcers within 12-24 hrs (9,10,11). This model is frequently used for investigation on the physiopathology of peptic ulcer disease. It is known that mucosal microcirculatory dysfunction and reduction of mucus secretion play an important role in the development of cysteamine-induced gastroduodenal ulcers, as well as in the generation of peptic ulcer disease (7,9,12,13). Thus, in female rats, we examined the effects of ovariectomy, 17β-estradiol and progesterone pretreatment on the severity of cysteamine-induced lesions in the stomach and the duodenum as assessed by measuring the extent of lesions, the microvascular permeability and the production of gastroduodenal mucus. Another ulcerogenic drug, indomethacin was also used, as it is known to induce gastroduodenal damage and delay the healing of ulcers induced by chemical factors (14,15).

Materials and Methods

Adult female Sprague Dawley rats (purchased from Charles River, Italy) weighing 200-250 g were used throughout all experiments. Rats were housed 4-5 *per* cage in a room under constant conditions of illumination (lights on between 06.00 and 18.00 hrs), temperature (22 ± 2 °C) and humidity (20-25%). Standard diet and tap water were available *ab libitum*. After a week of habituation in the facilities, animals were admitted to the experimental sessions. All experiments were carried out according to the European Communities Council Directive 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used. A group of animals was subjected to bilateral ovariectomy under ether anesthesia 2 weeks before the beginning of pharmacological treatment. Control animals were subjected to sham-operation under the same experimental conditions.

Two different experiments were performed. The first experiment involved ovariectomized and sham-operated female rats: on these animals, the extent of gastroduodenal lesions, plasma leakage and gastroduodenal mucus levels were measured after cysteamine or indomethacin treatment as described below. The same parameters were examined in the second experiment, where the influence of a pre-treatment with female sex steroids on cysteamine- or indomethacin-induced gastroduodenal effects was studied in intact female rats.

Ovariectomized and sham-operated female rats were injected acutely with 400 mg/kg cysteamine or repeatedly with 2 mg/kg/day indomethacin for 7 days. Drugs were provided by Sigma (U.S.A.) dissolved in saline and injected subcutaneously (s.c.). Before receiving the acute treatment with cysteamine or the last injection of indomethacin, animals were deprived of food for 24 hrs but received water *ad libitum*. They were observed for 10 hrs and sacrificed by ether overdose.

Intact female rats were treated with progesterone (as metoxyprogesterone acetate) or 17β-estradiol benzoate dissolved in olive oil and injected intramuscularly (i.m.) at the doses of 10, 25 or 50 mg/kg/week and 1, 2.5 or 5 mg/kg/week, respectively. These doses were selected being active in previous experiments (7,8). In a group of animals, the injections were made a week before the acute s.c. administration of cysteamine 400 mg/kg dissolved in saline. Other animals were injected with progesterone or 17β-estradiol contemporary with the first dose of 2 mg/kg/day indomethacin dissolved in saline and injected daily for 7 days. The total volume of solutions injected did not exceed 0.5 ml. Control rats received the same amount of olive oil alone, as placebo. Metoxyprogesterone acetate and 17β-estradiol benzoate were provided by Sigma (USA).

After sacrifice of animals, their stomachs and duodena were removed, washed with saline, dried on a filter paper and were weighted, cut along the gastric lesser curvature and examined planimetrically with an illuminated magnifier (x3). The area of the total mucosa surface and the injured parts were measured, and the data were expressed as the % ratio of the injured/total area.

Plasma leakage of ^{159}I-labelled human serum albumin ([^{125}I]-HSA) was determined in the stomach and the duodenum, as an index of vascular endothelial damage. For this purpose, [^{125}I]-HSA (2 μCi/kg) was administered intravenously (i.v.) 2 hrs before autopsy. Blood was collected from the abdominal aorta into syringes containing trisodium citrate (final concentration equal to 0.318%) and centrifuged (10,000 g x 4 min, 4° C). The [^{125}I]-HSA content in plasma and segments of gastric and duodenal tissue was determined in a gamma-spectometer (Nuclear Enterprises NE 1600). The albumin content in the stomach and the duodenum was calculated taking into account any changes in gastroduodenal blood volume as described previously (16). Values from tissues of control rats were subtracted from the values of treated animals, and the data were expressed as plasma leakage (μl plasma/g wet tissue). ^{125}I-labelled human serum albumin was obtained from Izinta (Hungary).

The measurement of gastric and duodenal barrier mucus levels was performed using the method described by Corne et al. (17). Briefly, the glandular portion of the stomach and the duodenum (prepared as described above) were excised and immersed for 2 hr in 0.1 % Alcian blue in a 0.16 mol/l sucrose solution buffered with 0.05 mol/l sodium acetate (pH adjusted to 5.8 with HCl). The unbound dye was removed by two subsequent washing of 15 and 45 min in 0.25 mol/l sucrose; the mucus-bound dye was eluted by immersing the stomach in a 0.5 mol/l $MgCl_2$ solution for 2 hr. The solution thus was shaken with diethyl ether and the optical density of the aqueous

phase was measured at 605 nm in a spectrophotometer Hitachi 150-20. The quantity of Alcian blue, extracted *per* g of wet glandular tissue, was then calculated from standard curves.

Plasma 17β-estradiol levels were measured after ovariectomy by a radioimmunoassay method that has been described previously (3).

The data were expressed as mean±SEM from seven rats *per* each experimental group. Statistical analysis of data was made using the two-way ANOVA and the post hoc Dunnett's test for multiple comparisons. The Student's t-test for paired data was used to analyze the values of plasma

TABLE I

Effects of ovariectomy on gastric and duodenal lesions, plasma leakage and decrease of gastroduodenal mucus induced by cysteamine (400 mg/kg, s.c.) or indomethacin (2 mg/kg/day for 7 days) in female rats.

Groups	Extent of lesions (%)	Δ Plasma leakage (μl/g)	Mucus-bound dye (μg dye/g tissue)
1. Sham-operation + saline (7)	0.00±0.00	0.00±0.00	380.1±13.0
2. Sham-operation + cysteamine (7)	10.51±0.16*	160.1±11.0*	271.1±12.1*
3. Sham-operation + indomethacin (7)	6.00±0.10*	110.1±10.1*	301.1±14.5*
4. Ovariectomy + saline (7)	0.00±0.00	0.00±0.00	478.1±24.3*
5. Ovariectomy + cysteamine (7)	2.89±0.01#	60.1±5.71#	367.2±34.4#
6. Ovariectomy + indomethacin (7)	1.42±0.01§	15.1±1.21§	369.2±21.1§

Values are percentage. In parentheses in indicated the number of animals *per* each group.
*Significant difference vs. group 1 ($p<0.01$, Dunnett's test for multiple comparisons)
#Significant difference vs. group 2 ($p<0.05$, Dunnett's test for multiple comparisons)
§Significant difference vs. group 3 ($p<0.05$, Dunnett's test for multiple comparisons).

17β-estradiol levels in ovariectomized animals compared to intact controls. A p level of 0.05 or less was considered as indicative of a significant difference.

Results

Ovariectomy performed two weeks before cysteamine challenge decreased plasma 17β-estradiol levels (from 182.3±9.8 to 86.8±5.3 pmol/l, n=6, $p<0.001$, Student's t-test).

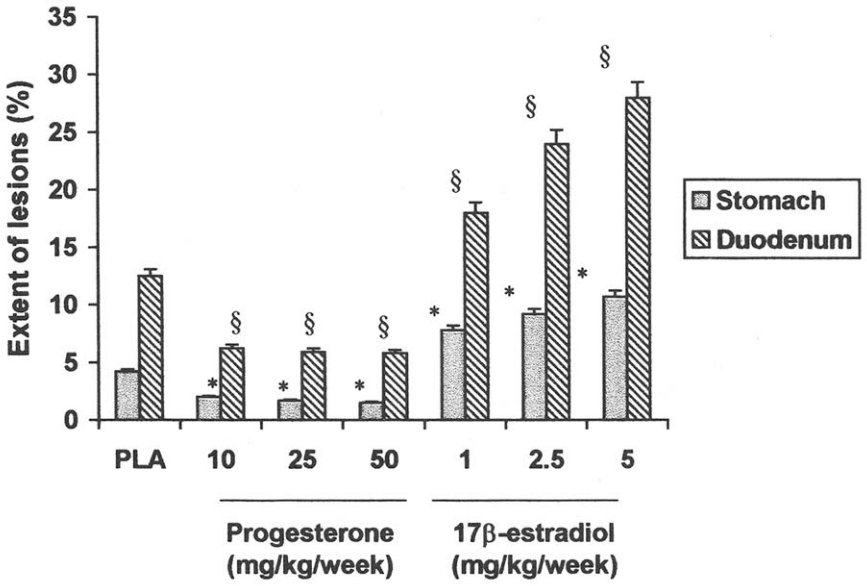

Fig. 1

Effects of treatment with progesterone (10, 25 or 50 mg/kg/week, i.m.) or 17β-estradiol (1, 2.5 or 5 mg/kg/week, i.m.) on the extent of lesions induced by cysteamine (400 mg/kg, s.c.) in the stomach and in the duodenum of intact female rats. Values are mean±SEM. The number of animals *per* each group is 7. Two-way ANOVA revealed a significant treatment effect, $F(1,111) = 166.5$ ($p<0.05$). *Significantly different as compared to stomach of rats treated with placebo (PLA) before the injection of cysteamine ($p<0.05$, post-hoc Dunnett's test for multiple comparisons). §Significantly different as compared to duodenum of rats treated with placebo (PLA) before the injection of cysteamine ($p<0.05$, post-hoc Dunnett's test for multiple comparisons).

As expected, administration of cysteamine 400 mg/kg induced macroscopic gastroduodenal mucosa injury in placebo-treated controls. However, cysteamine-induced gastroduodenal damage appeared to be diminished in ovariectomized rats (Table 1). The pretreatment with progesterone (10-50 mg/kg/week) also reduced the severity of cysteamine-induced gastroduodenal lesions in a dose-dependent manner (Fig. 1). In contrast, the pretreatment with 17β-estradiol (1-5 mg/kg/week) dose-dependently augmented gastric and duodenal macroscopic lesions.

The microvascular permeability in the stomach and the duodenum appeared to be increased in cysteamine-injected rats. This drug effect was found to be inhibited in ovariectomized rats (Table 1) and in animals pretreated with progesterone (Figure 2). In contrast, the pretreatment with 17β-estradiol further increased the cysteamine effect. Progesterone and 17β-estradiol appeared to be effective in a dose-dependent manner.

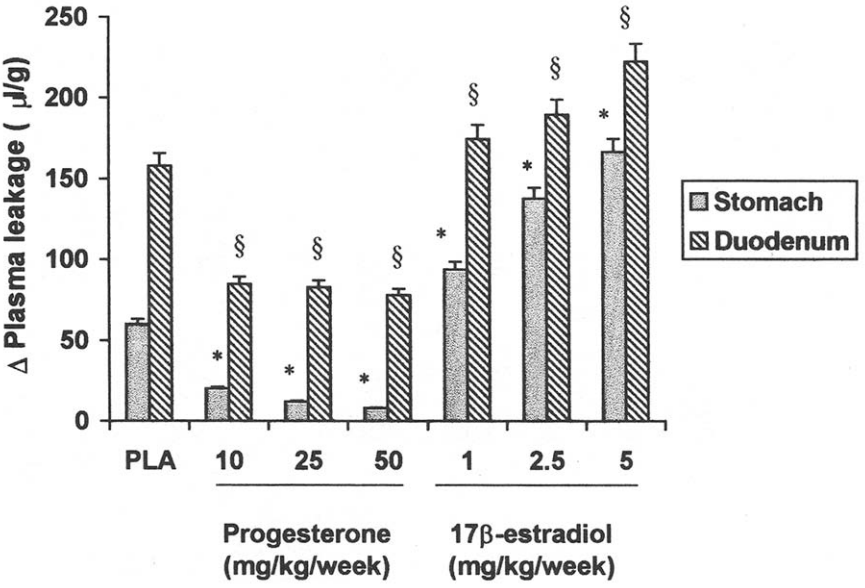

Fig. 2

Effects of treatment with progesterone (10, 25 or 50 mg/kg/week, i.m.) or 17β-estradiol (1, 2.5 or 5 mg/kg/week, i.m.) on plasma leakage induced by cysteamine (400 mg/kg, s.c.) in the stomach and in the duodenum of intact female rats. Values are mean±SEM. The number of animals *per* each group is 7. Two-way ANOVA revealed a significant treatment effect, $F(1,111) = 171.5$ ($p<0.05$). *Significantly different as compared to stomach of rats treated with placebo (PLA) before the injection of cysteamine ($p<0.05$, post-hoc Dunnett's test for multiple comparisons). §Significantly different as compared to duodenum of rats treated with placebo (PLA) before the injection of cysteamine ($p<0.05$, post-hoc Dunnett's test for multiple comparisons).

Acute cysteamine injection decreased gastroduodenal mucus levels. Both ovariectomy (Table 1) and the pretreatment with progesterone inhibited this effect of cysteamine (Figure 3). Progesterone was effective at all doses used and in a dose-dependent manner. In contrast, 17β-estradiol dose-dependently further reduced the level of gastroduodenal mucus.

In different experiments, the treatment with indomethacin caused macroscopic gastroduodenal damage (Table 1). Ovariectomy decreased indomethacin-induced gastroduodenal injury. Furthermore, the injection of 17β-estradiol (1-5 mg/kg/week) did not affect gastroduodenal damage, while treatment with progesterone (10-50 mg/kg/week) protected against indomethacin-provoked mucosa ulcers (Table 2).

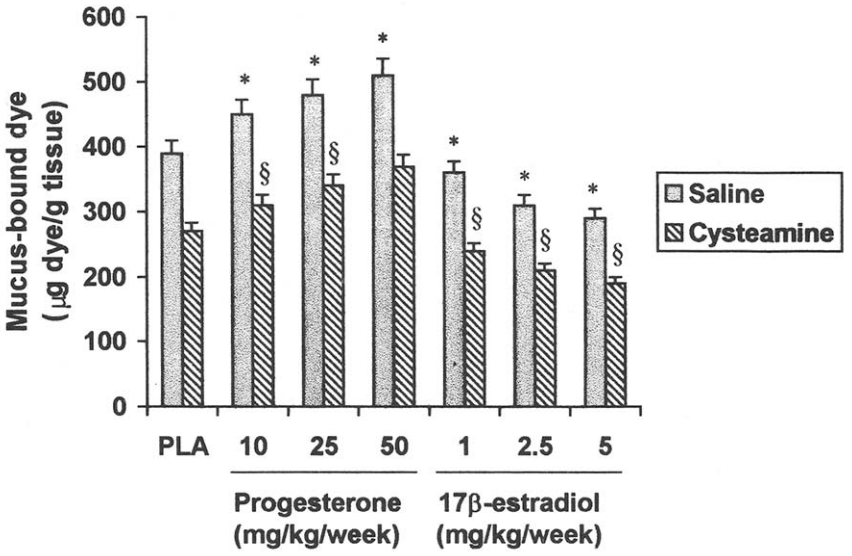

Fig. 3

Effects of treatment with progesterone (10, 25 or 50 mg/kg/week, i.m.) or 17β-estradiol (1, 2.5 or 5 mg/kg/week, i.m.) on gastroduodenal mucus level induced by cysteamine (400 mg/kg, s.c.) in the stomach and in the duodenum of intact female rats. Values are mean±SEM. The number of animals *per* each group is 7. Two-way ANOVA revealed a significant treatment effect, $F(1,111) = 156.5$ ($p<0.05$). *Significantly different as compared to control rats treated with placebo (PLA) before the injection of saline ($p<0.05$, post-hoc Dunnett's test for multiple comparisons). §Significantly different as compared to rats treated with placebo (PLA) before the injection of cysteamine ($p<0.05$, post-hoc Dunnett's test for multiple comparison).

Discussion

This study confirms the previous observation (3) that following ovariectomy the gastroduodenal mucosa is less sensitive to ulcerogenic stimuli. The reduction by ovariectomy of cysteamine- and indomethacin-induced ulcers of the stomach and the duodenum might relate to the decrease of plasma 17β-estradiol levels, since mucosal damage induced by cysteamine was increased by exogenous administration of the hormone into intact female rats.

In contrast, administration of progesterone protected the gastroduodenal mucosa against ulcerogenic treatment with cysteamine or indomethacin. This last finding is in agreement with recent data showing that an increase of endogenous progesterone levels by early pregnancy or the administration of exogenous progesterone decrease the vulnerability of gastroduodenal mucosa to cysteamine (7). Interestingly, progesterone acts as a protective factor also in male rats. The present findings may thus suggest an involvement of female sex steroids in the pathogenesis

TABLE II

Effects of a pretreatment with progesterone (10-50 mg/kg/week, s.c.) or 17β-estradiol (1-5 mg/kg/week, s.c.) on gastric and duodenal lesions induced by indomethacin (2 mg/kg/day for 7 days) in intact female rats.

Groups	Extent of lesions (%)
1. Saline + indomethacin (7)	5.95±0.11
2. 17β-estradiol (1 mg/kg/week) + indomethacin (7)	4.47±0.10
3. 17β-estradiol (2.5 mg/kg/week) + indomethacin (7)	4.41±0.11
4. 17β-estradiol (5 mg/kg/week) + indomethacin (7)	6.61±0.13
5. Progesterone (10 mg/kg/week) + indomethacin (7)	3.28±0.09*
6. Progesterone (25 mg/kg/week) + indomethacin (7)	2.87±0.05*
7. Progesterone (50 mg/kg/week) + indomethacin (7)	2.35±0.04*

Values are percentage. In parentheses in indicated the number of animals *per* each group.
*Significant difference vs. group 1 ($p<0.01$, Dunnett's test for multiple comparisons)

of gastroduodenal ulcers.

The sex-dependent difference of peptic ulcer disease and the beneficial role of pregnancy in the generation of gastroduodenal mucosa damage is well established in humans (1,2) and suggests a physiopathological role of endogenous sex steroids in ulcers. However, only few data are available in literature dealing with the effects of these hormones on gastroduodenal mucosa. In the present study ovariectomy was followed in cysteamine-treated animals by increased gastoduodenal mucus level that is an important protective factor in the pathogenesis of peptic ulcer (18,19). Also, increased mucus secretion was observed by the administration of exogenous progesterone. This finding is possibly consistent with the data showing that increased release of endogenous progesterone enhanced gastroduodenal mucus secretion and attenuates cysteamine-induced ulcers (7). In contrast, treatment with 17β-estradiol was shown to decrease gastroduodenal mucus production and worsen cysteamine-induced mucosa lesions. Thus, it appears that the modulation of mucus production in the stomach and the duodenum by female sex steroids might play an important role in the pathogenesis of peptic ulcers.

It is known that an increase of gastric parietal mass plays a beneficial role in the development of mucosa lesions following various ulcerogenic treatments (20). Furthermore, it has been shown that ovariectomy enhances gastric parietal cell mass (21) and the number of mucosal gastrin receptors that may be involved in hypertrophic responses of the gastric mucosa (22). This elevation in parietal cell number may be a possible explanation of the present findings showing that ovariectomy protects the gastric and duodenal mucosa against cysteamine- or indomethacin-

induced injury. Indeed, earlier animal studies have shown that female sex hormones may influence gastric acid secretion (23,24). Furthermore, although both cysteamine and indomethacin may induce gastroduodenal inflammation, no data exist on the influence of female sex hormones on histological tissue integrity and/or myeloperoxidase activity in the gastroduodenal mucosa of female ovariectomized rats compared to intact animals. For a better understanding of these results and to explain the aggressive role of 17β-estradiol, it should be mentioned here that in other studies it was demonstrated that administration of estrogens leads to the inactivation of prostaglandins (25) that are known to have a key importance in the defense mechanisms of the gastroduodenal mucosa (16,26).

In contrast to that found in experiments where cysteamine was used, the administration of 17β-estradiol did not modify the gastroduodenal damage induced by indomethacin in intact female rats. Although this finding seems not to be consistent with the general concept of an aggressive role played by 17β-estradiol on gastroduodenal mucosa, it should be noted that in our experiments a depot preparation was applied for this hormone that was administered in olive oil solution a week prior to sacrifice. In agreement with other studies (14), indomethacin was administered s.c. in a daily dose regimen of 2 mg/kg. After 7 days of treatment, the drug caused a sustained gastroduodenal damage. In contrast, cysteamine was injected at the dose of 400 mg/kg in a single bolus, a week after the hormone administration. Thus, it is possible that the particular preparation of 17β-estradiol and its mode of administration may have modified the pharmacological activity of the hormone.

The present study shows that cysteamine causes microvascular plasma leakage, considered as an index of vascular endothelial damage. These findings are consistent with the previous suggestion that aggressive vascular factors are involved in the generation of cysteamine-induced gastroduodenal ulcers (13). It is also well established that microcirculatory injury and the consequent hypoxia are among those factors which are considered to lead to gastroduodenal ulcers (16). Furthermore, reduction of plasma 17β-estradiol levels following ovariectomy leaded to attenuation of cysteamine-induced microvascular leakage. In contrast, 17β-estradiol administration aggravated this phenomenon. Interestingly, great evidence exists that estrogens interfere with factors involved in blood coagulation (27). Although a clear sense of the net effect of physiological and pharmacological doses of estrogens on coagulation has not yet emerged (28), a focus should be given here that the vascular endothelium express estrogen receptors and a number of special estrogen derivatives can be used as hemostatic agents (27).

Consistently with human observations, these findings show that female sex steroids are involved in the physiopathological mechanisms of gastroduodenal ulcers. It seems that they play a dual role in cysteamine- and indomethacin-induced gastroduodenal mucosa lesions by modulating microvascular permeability and mucus production. Progesterone may exert a protective action, in contrast to 17β-estradiol that exerts an aggressive action. The use of selective antagonists for these hormones may confirm their role in the physiopathology of peptic ulcers.

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