ELSEVIER

European Journal of Pharmacology 337 (1997) 275–278

ejp

Short communication

Damaging actions of testosterone on cysteamine-induced gastroduodenal ulceration and vascular leakage in the rat

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Received 30 June 1997; revised 6 August 1997; accepted 12 August 1997

Abstract

The sexual dimorphism of gastroduodenal ulceration is suggested on the basis of clinical and experimental observations. This difference probably relates to the actions of endogenous sexual steroids. In the present study, the role of testosterone was evaluated in the generation of gastroduodenal mucosal injury provoked by cysteamine (400 mg/kg, s.c.). We found that macroscopic mucosal damage and microvascular ^{125}I-human serum albumin leakage (2 μCi/kg, i.v.) developed in the stomach and duodenum of male rats 24 h after the administration of cysteamine. This mucosal injury was prevented by orchidectomy and by the pretreatment with the antiandrogen, cyproterone acetate (12 mg/kg per day for 8 consecutive days). It was also shown that pretreatment with testosterone (4–20 mg/kg per week) dose-dependently aggravated cysteamine-induced gastroduodenal mucosal injury. Our results thus suggest an aggressive role of testosterone in the generation of cysteamine-induced gastroduodenal ulceration. © 1997 Elsevier Science B.V.

Keywords: Gastroduodenal ulceration; Gastroduodenal mucosa; Cysteamine; Testosterone; Sexual dimorphism; Sexual steroid; Microcirculation; Microvascular leakage

1. Introduction

In animal studies, several observations suggest that sex hormones possibly play a role in the generation of various experimental ulcers. Pregnancy and lactation in rats markedly reduce steroid- and cysteamine-induced gastroduodenal lesions (Kelly and Robert, 1969; Montoneri and Drago, 1997). Moreover, the sexual dimorphism of experimental mucosal damage has also been described. For example, restraint and activity stress induce more severe lesions in the stomach of female rats (Robert et al., 1966; Robert and Nezamis, 1975). In contrast, gastric mucosal erosions were shown to be enhanced in males in comparison with females following oral administration of ethanol (László et al., 1992, 1993).

In human studies, the low incidence of peptic ulcers among pregnant women was found (Michaletz Onody, 1992). During pregnancy, hemorrhage or/and perforation from gastroduodenal ulceration has shown to be rare during pregnancy compared to its higher incidence in puerperium (Aston et al., 1991). Finally, in the fertile age, peptic ulcer disease is more frequent in men than in women (Aston et al., 1991; Michaletz Onody, 1992)

It is known that following the administration of cysteamine gastric mucosal lesions and duodenal ulceration occurs within 12–24 h (Szabó et al., 1976; Bernardini et al., 1989). This model is frequently used for the investigation of the pathophysiology of peptic ulcer disease. In the present study, the role of endogenous and exogenous testosterone has been evaluated in the generation of gastric and duodenal ulceration and vascular permeability following the administration of cysteamine.

2. Materials and methods

2.1. Induction of gastroduodenal mucosal injury

Male Wistar rats with an initial body weight of 220–250 g were used. Gastric and duodenal mucosal lesions were provoked by the single administration of cysteamine (Sigma, St. Louis, MO, USA; 400 mg/kg, s.c.) 24 h later. During this period the animals were deprived of food, but

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received water ad libitum. For the determination of microvascular leakage, under light ether anaesthesia, [^{125}I]human serum albumin (HSA; Izinta, Budapest, Hungary; 2 μCi/kg, into the tail vein) was administered 2 h before autopsy.

2.2. Macroscopic evaluation

Twenty-four hours after the administration of cysteamine, the animals were sacrificed and mucosal lesions were analyzed planimetrically. The area of the total mucosal surface and the lesioned parts were measured in the whole glandular stomach and in the duodenum (on a standard 15–17 mm long piece from the pylorus). Data were expressed as the ratio of the lesioned/total area in %.

2.3. Albumin leakage

As a measure of vascular endothelial damage, leakage of [^{125}I]HSA was determined in the stomach and duodenum. Immediately before autopsy, under ether anaesthesia, blood was collected from the abdominal aorta into syringes containing trisodium citrate (final concentration 0.318%) and centrifuged ($10\,000 \times g$, 10 min, 4°C). The [^{125}I]HSA content of the stomach, duodenum and plasma was determined in a gamma spectrometer (Nuclear Enterprises NE 1600) and the albumin content in gastroduodenal tissue was calculated as described previously (Boughton-Smith et al., 1993). Control values (from rats that had received saline) were subtracted from the treated values and the data were expressed as Δ albumin leakage in μl/g tissue.

2.4. Groups and treatments

Under pentobarbitone (Serva, Heidelberg, Germany; 40 mg/kg, i.p.) anaesthesia rats were orchidectomized 2 weeks before the administration of cysteamine. After orchidectomy, the plasma testosterone level was checked by a radioimmunoassay method described previously (László et al., 1992). In further experiments, the antiandrogen, cyproterone acetate (Androcur, Schering, Berlin, Germany; 12 mg/kg per day, p.o.) was administered into male rats during 8 consecutive days before cysteamine exposition. This dose of cyproterone acetate has been shown previously to reduce plasma testosterone level (Pávó et al., 1995). Finally, another group of male rats received a depot injection of testosterone phenylpropionate (Retandrol, Richter, Budapest, Hungary; 4–20 mg/kg, i.m.) one week before cysteamine administration.

2.5. Statistics

The results were analyzed using the nonparallel Mann–Whitney *U*-test, and differences were taken as significant when the probability level was less than 5%.

3. Results

3.1. Provocation of mucosal damage

Administration of cysteamine (400 mg/kg, s.c.) provoked macroscopic mucosal injury involving $5.4 \pm 0.3\%$ and $15.3 \pm 1.8\%$ of the stomach and duodenum, respectively ($n = 8$, $P < 0.001$) over 24 h in control male rats. In the duodenum and the stomach, significant albumin leakage was also detected 24 h after cysteamine administration, being $\Delta 74 \pm 8$ and $\Delta 112 \pm 15$ μl/g tissue, respectively ($n = 8$, $P < 0.001$) as shown in Figs. 1 and 2.

3.2. Effects of orchidectomy and antiandrogen treatment on mucosal damage

We found that cysteamine-induced macroscopic mucosal injury was inhibited by $94 \pm 2\%$ in the stomach ($n = 5$, $P < 0.001$) and by $96 \pm 3\%$ in the duodenum ($n = 5$, $p < 0.001$) following orchidectomy (Figs. 1 and 2). In orchidectomized animals, gastric and duodenal albumin leakage was also shown to be reduced (by $86 \pm 12\%$ and $73 \pm 13\%$, respectively, $n = 5$, $P < 0.001$) 24 h after cysteamine administration (Figs. 1 and 2). In addition, the plasma testosterone level decreased from 13.4 ± 2.1 to 3.8 ± 0.8 nmol/l ($n = 6$, $P < 0.001$) after orchidectomy.

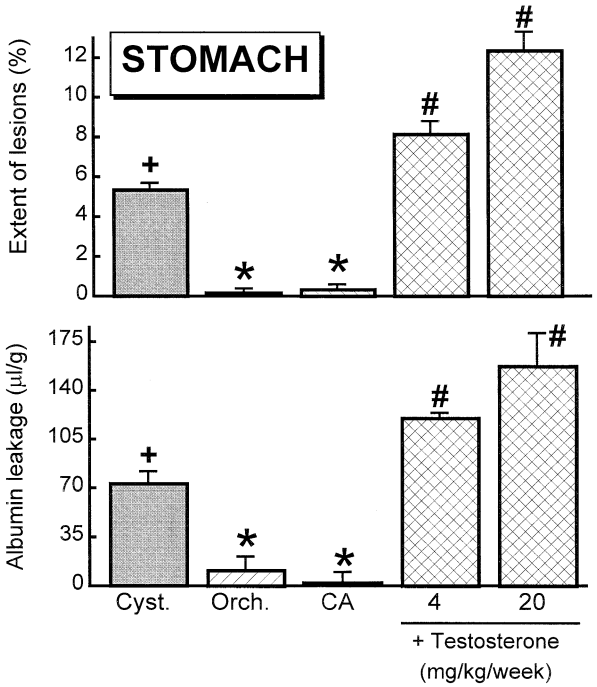

Fig. 1. Induction by cysteamine (Cyst., 400 mg/kg, s.c.) of gastric mucosal macroscopic lesions (upper panel) and microvascular albumin leakage (lower panel) over 24 h in the male rat. Inhibition of cysteamine-induced mucosal injury by orchidectomy (Orch.), and the antiandrogen, cyproterone acetate (CA, 12 mg/kg per day, p.o.), and its potentiation by exogenous testosterone (4–20 mg/kg per week). Data are expressed as the mean ± S.E.M. of minimum 5 rats per group; statistical significance is shown as mucosal damage provoked by cysteamine: $^{+}P < 0.05$, inhibition of cysteamine-induced damage: $^{*}P < 0.05$, potentiation of cysteamine-induced damage: $^{\#}P < 0.05$.

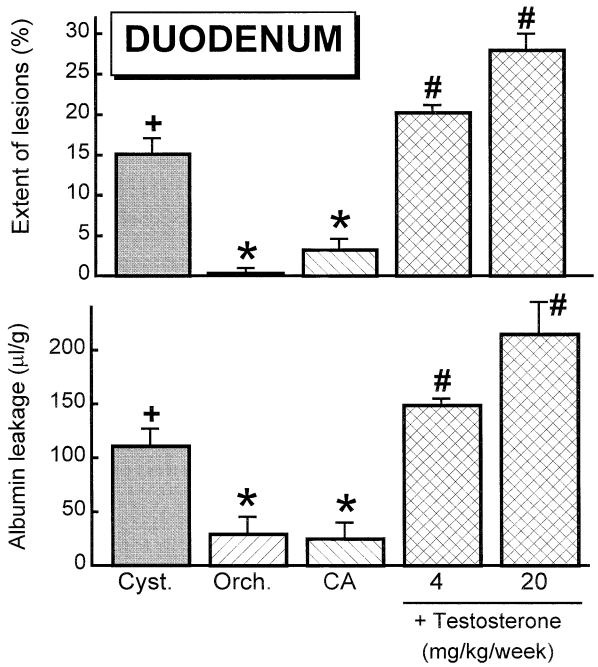

Fig. 2. Induction by cysteamine (Cyst., 400 mg/kg, s.c.) of duodenal mucosal macroscopic lesions (upper panel) and microvascular albumin leakage (lower panel) over 24 h in the male rat. Inhibition of cysteamine-induced mucosal injury by orchidectomy (Orch.), and the antiandrogen, cyproterone acetate (CA, 12 mg/kg per day, p.o.), and its potentiation by exogenous testosterone (4–20 mg/kg per week). Data are expressed as the mean ± S.E.M. of minimum 5 rats per group; statistical significance is shown as mucosal damage provoked by cysteamine: $^{+}P < 0.05$, inhibition of cysteamine-induced damage: $^{*}P < 0.05$, potentiation of cysteamine-induced damage: $^{\#}P < 0.05$.

Pretreatment with cyproterone acetate (12 mg/kg per day for 8 consecutive days) reduced significantly cysteamine-provoked macroscopic mucosal lesions and albumin leakage in the stomach (by 93 ± 4% and by 99 ± 1%, respectively, $n = 6$, $P < 0.001$) as shown in Fig. 1. We found similar protection against cysteamine-induced mucosal injury and albumin leakage in the duodenum of antiandrogen-treated male rats (Fig. 2).

3.3. Effects of exogenous testosterone on mucosal damage

In contrast to the beneficial actions of orchidectomy or cyproterone acetate, testosterone administered exogenously (4–20 mg/kg per week) dose-dependently augmented macroscopic mucosal injury and albumin leakage in the stomach (by 130 ± 17% and 114 ± 31%, respectively, $n = 6$, $P < 0.001$) and in the duodenum (by 87 ± 12% and 93 ± 26%, respectively, $n = 6$, $P < 0.001$) induced by cysteamine (Figs. 1 and 2).

4. Discussion

In the present study, the influence of testosterone has been evaluated in the generation of gastroduodenal mucosal injury provoked by cysteamine. We found that the inhibition of endogenous testosterone synthesis by orchidectomy and the antiandrogen, cyproterone acetate treatment lead to protection against macroscopic mucosal injury and microvascular damage provoked by cysteamine in the stomach and duodenum. In contrast, testosterone, when it was administered exogenously, aggravated cysteamine-induced gastroduodenal mucosal damage. These findings correspond with the theory that sexual steroids play a modulatory role in the development of ulceration of the gastroduodenal mucosa, and might possibly confirm those clinical observations that peptic ulcer disease is more frequent in men than in women (Aston et al., 1991; Michaletz Onody, 1992).

Although the sex-difference in peptic ulcer disease is well-known, only very few attempts can be found in the literature to evaluate the effects of sexual steroids on the physiology and pathology of the gastroduodenal mucosa. In a recent study, the elevation of endogenous progesterone levels (in early pregnant rats) and the administration of progesterone increased mucus thickness in the stomach and duodenum, and protected against cysteamine-induced mucosal ulceration (Montoneri and Drago, 1997). Getting closer to a better understanding of our present study, in the experiments of Adeniyi (1991) reduced gastric acid secretion has been demonstrated in orchidectomized rats relative to intact males. Since an increased gastric acid output is revealed to be an important factor in development of cysteamine-induced ulceration (Szabó et al., 1976; Bernardini et al., 1989), a decreased gastric acid secretion may explain the protection against gastroduodenal ulceration in our testosterone synthase inhibited animals.

Another possible pathway of the pathogenetic action of testosterone in cysteamine-induced gastroduodenal ulceration is its interaction with the endogenous vasoconstrictor vasopressin. In recent works, endogenous vasopressin has been demonstrated to play an aggressive role towards the gastroduodenal mucosa, since vasopressin-deficient rats (Brattleboro homozygous strain) and humans (patients with diabetes insipidus) are shown to be resistant against gastroduodenal ulceration (László et al., 1994, 1997). On the other hand, it was shown that exogenous testosterone increases the number of vasopressin receptors, while orhidectomy decreased vasopressin binding sites and plasma vasopressin level (Pávó et al., 1995). Moreover, administration of testosterone augmented blood pressure elevation following vasopressin injection (László et al., 1991). Finally, it should be mentioned here that the reduction of mesenteric circulation has been suggested to be involved in the pathogenesis of cysteamine-induced mucosal injury (Bernardini et al., 1989). Taking these results together, endogenous testosterone may have such harmful microvascular effects (we found in the present study an increased albumin leakage) which might possibly injure gastroduodenal mucosa by mediating the damaging actions of endogenous vasopressin.

In conclusion, endogenous testosterone seems to have significant damaging actions towards the gastroduodenal mucosa during the development of ulceration provoked by cysteamine. It can possibly mean that testosterone might play a role in the sexual dimorphism observed in peptic ulcer disease. It is likely that further studies are needed for the evaluation of the influence of oestrogens in the generation of mucosal damage of the gut.

Acknowledgements

This work was supported by The Hungarian Ministry of Higher Education (FKFP 0045/1997) and by The Hungarian Ministry of Social Welfare (T-02 642/1996). Ferenc László was a short term research fellow at Inst. of Pharmacology, Univ. of Catania.

References

Adeniyi, K.O., 1991. Gastric acid secretion and parietal cell mass: Effect of sex hormones. Gastroenterology 101, 66–69.

Aston, N.O., Kalaichadran, S., Carr, J.V., 1991. Duodenal ulcer hemorrhage in the puerperium. Can. J. Surg. 34, 482–483.

Bernardini, M.C., Blandizzi, C., Morini, G., Chiavarini, M., Impicciatore, M., Del Tacca, M., 1989. Pirenzepine prevents cysteamine-induced formation of gastroduodenal ulcers and reduction of mesenteric circulation. Arch. Int. Pharmacodyn. 302, 242–254.

Boughton-Smith, N.K., Evans, S.M., László, F., Whittle, B.J.R., Moncada, S., 1993. The induction of nitric oxide synthase and intestinal vascular permeability by endotoxin in the rat. Br. J. Pharmacol. 109, 1189–1195.

Kelly, P., Robert, A., 1969. Inhibition by pregnancy and lactation of steroid-induced ulcers in the rat. Gastroenterology 56, 24–29.

László, F.A., László, F., De Wied, D., 1991. Pharmacology and clinical perspectives of vasopressin antagonists. Pharmacol. Rev. 43, 73–108.

László, F., Amani, E., Varga, Cs., László, F.A., 1992. Influence of sex hormones on ethanol-induced gastric haemorrhagic erosions in rats. Acta Physiol. Hung. 80, 289–292.

László, F., Amani, E., Karácsony, G., Szabó, E., Rengei, B., Varga, Cs., László, F.A., 1993. The modulatory role of endogenous vasopressin in the phenomenon that orally administered ethanol generates more severe gastric erosions in male than in female rats. Ann. N.Y. Acad. Sci. 689, 623–626.

László, F., Karácsony, G., Pávó, I., Varga, Cs., Rojik, I., László, F.A., 1994. Aggressive role of vasopressin in development of different gastric lesions in rats. Eur. J. Pharmacol. 258, 15–22.

László, F., Szepes, Z., Varga, Cs., László, F.A., 1997. Deleterious actions of vasopressin in gastroduodenal ulceration: Clinical and experimental observations. Scand. J. Gastroenterol., in press.

Michaletz Onody, P.A., 1992. Peptic ulcer disease in pregnancy. Gastroenterol. Clin. North Am. 21, 817–826.

Montoneri, C., Drago, F., 1997. Effects of pregnancy on cysteamine-induced peptic ulcers: Role of progesterone. Dig. Dis. Sci., in press.

Pávó, I., Varga, Cs., Szûcs, M., László, F., Szécsi, M., Gardi, J., László, F.A., 1995. Effects of testosterone on rat renal medullary vasopressin receptor concentration and the antidiuretic response. Life Sci. 56, 1215–1222.

Robert, A., Nezamis, J.E., 1975. Exertion ulcers. In: Gheurgiu, T. (Ed.), Experimental Ulcer. Gerhard Witzstrock, Baden-Baden, pp. 77–85.

Robert, A., Phillips, J.P., Nezamis, J.E., 1966. Production, by restraint, of gastric ulcers and of hydrothorax in the rat. Gastroenterology 51, 75–80.

Szabó, S., Reynolds, E.S., Lichtenberger, L.M., Dzan, V.J., 1976. Pathogenesis of duodenal ulcer. Gastric hyperacidity caused by propionitrile and cysteamine in rats. Res. Commun. Chem. Pathol. Pharmacol. 16, 311–322.