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European Journal of Pharmacology 392 (2000) R5–R7

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Rapid communication

Nitric oxide-mediated mucus hypersecretion protects the stomach of ovariectomized rats

Éva Morschl [a], Ildikó Bretus [a], Imre Pávó [b], Lajos Topa [c], Zsóka Weiszhar [d], Ferenc László [d,*]

[a] *First Department of Medicine, Albert Szent-Györgyi Medical University, Szeged, Hungary*
[b] *Endocrine Unit, Albert Szent-Györgyi Medical University, Szeged, Hungary*
[c] *Second Department of Medicine, St. Imre University Teaching Hospital, Budapest, Hungary*
[d] *Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony u. 43, H-1083 Budapest, Hungary*

Received 19 January 2000; accepted 25 January 2000

Abstract

The actions of ovariectomy on nitric oxide synthase (assessed by the citrullin assay), mucus secretion (assessed by the Alcyan blue technique) and ulcerogenic response (indomethacin (30 mg kg^{-1}, s.c., 4 h) or cysteamine (400 mg kg^{-1}, s.c., 24 h)) were studied in the female rat stomach. Ovariectomy increased nitric oxide synthase and mucus secretion, and decreased the severity of lesions, effects reversed by the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}, s.c., 4 h before measurements). Therefore, estrogen-deficiency protects the gastric mucosa by nitric oxide (NO)-mediated mucus hypersecretion.

Keywords: Nitric oxide (NO); Gastric mucus; Estrogen

The sexual dimorphism of gastric or/and duodenal ulceration is well known. Clinical and experimental observations suggest that sexual steroids have a key importance in the regulation of the defensive mechanisms of the gastroduodenal mucosa (Robert and Kauffman, 1989; László et al., 1997; Drago et al., 1999). In earlier studies, gonadectomy decreased the severity of gastric mucosal injury in various models of ulceration (László et al., 1997; Drago et al., 1999). It is also well established that numerous aggressive and protective factors affect the gastroduodenal mucosa, and an imbalance between them is pathogenic in the development of mucosal injury (Robert and Kauffman, 1989). Among the protective factors, gastroduodenal mucus plays a crucial role (Robert and Kauffman, 1989). Administration of nitric oxide (NO) donors stimulates mucus release from isolated gastric mucus-cell fraction (but not in parietal cells), and it has also been demonstrated that NO synthase is presented in these cells (Brown et al., 1992, 1993). Moreover, an increase in endogenous NO synthase activity in the gastric mucosa protected the stomach against damage (Tepperman et al., 1993). In the present study, the action of the estrogen-deficient state (i.e. ovariectomy) on gastric NO synthase activity, mucus secretion and on the susceptibility of the mucosa towards various ulcerogenic stimuli has been examined.

We used female Wistar rats (200–220 g). Sham operation or ovariectomy has been performed 1 month before the experiments. For provocation of gastric mucosal injury, indomethacin (30 mg kg^{-1}, s.c.) or cysteamine (400 mg kg^{-1}, s.c.) were administered, and the measurement (planimetry) of lesions has been performed 4 or 24 h later, respectively. The animals were starved for 24 h before (indomethacin) or during (cysteamine) lesion induction. For the evaluation of gastric mucosal NO synthase activity, we used the citrulline assay. The detailed description of the method can be found in the paper of Brown et al. (1992). For the measurement of gastric mucus level, we used the Alcyan blue technique, which methodological description can be found in details in the paper of Drago et al. (1999). For the investigation of the role of NO in our model, the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg kg^{-1}, s.c.) was administered into ovariectomized rats 4 h before any measurement. For the citrulline assay, L-[U-^{14}C]arginine monohydrochloride was obtained from Amersham International. All other com-

* Corresponding author. Tel.: +36-1-210-0819; fax: +36-1-210-0813.
E-mail address: laszlof@koki.hu (F. László).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00082-0

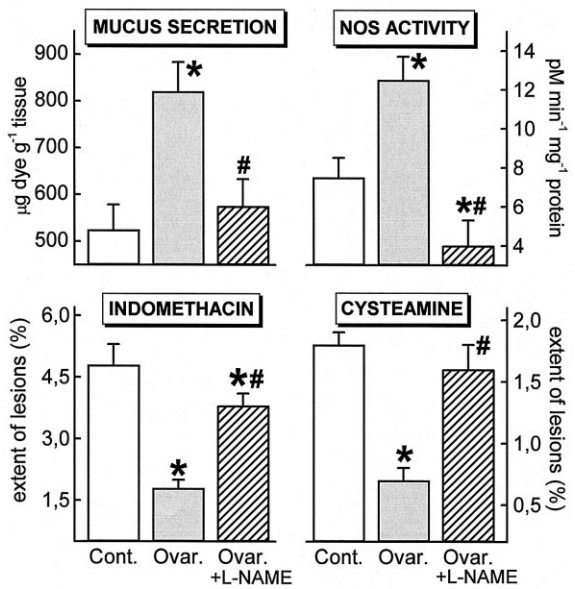

Fig. 1. Actions of ovariectomy (Ovar., grey columns) on mucus secretion (expressed as μg dye g^{-1} tissue), nitric oxide synthase activity (NOS, expressed as pM min^{-1} mg^{-1} protein) and lesion formation (expressed as extent of lesions in percent) provoked by indomethacin or cysteamine in the stomach of the female rat (Cont., open columns), and their reversal by the administration of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, hatched columns). Data are expressed as means ± S.E.M., where $n = 4–6$ rats in a group. $^*P < 0.05$ between Cont. and Ovar. groups; $^\#P < 0.05$ between Ovar. and Ovar. + L-NAME groups.

pounds were from Sigma. Data were analyzed with the Turkey–Kramer multiple comparisons test, where $P < 0.05$ was taken as significant.

We found that ovariectomy increased gastric NO synthase activity (by 96 ± 11%, $n = 6$, $P < 0.005$) and mucus secretion (by 57 ± 8%, $n = 5$, $P < 0.01$), and decreased the severity of lesions (by 63 ± 10% or by 61 ± 6% following indomethacin or cysteamine challenge, respectively; $n = 4–5$, $P < 0.001$). All these effects of ovariectomy were reversed by the administration of the NO synthase inhibitor, L-NAME (Fig. 1).

Our present findings confirm that gonadectomy improves the defensive mechanism of the stomach (László et al., 1997; Drago et al., 1999). Female sexual steroids have dual actions on the gastric mucosa. Progesterone prevents injury, since during early pregnancy (when progesterone level is high), a lower susceptibility of the stomach has been found (Montoneri and Drago, 1997). In contrast, estrogens augment damage, because of the gastric protection by ovariectomy and lactation, when the level of estrogens is low (Robert and Kauffman, 1989; Drago et al., 1999). These observations are apparently conflicting with the well-known experience, i.e. males are more prone to gastric ulceration than females, although their estrogen level is low. However, orchidectomy or testosterone antagonist revealed to protect against injury. This shows that in males, instead of harmful estrogens, testosterone plays an aggressive role towards the gastric mucosa (László et al., 1997). Testosterone may generate more severe gastric damage in males compared to females, since in males the protective progesterone level is also low.

In our study, an increased gastric mucosal NO synthase activity has been shown after ovariectomy. This elevated gastric NO production might have an important role in the protection against ulcerogenic challenge, since the administration of the NO synthase inhibitor, L-NAME restored mucosal damage in the ovariectomized rat to that level what could be observed in control females. An increased generation of NO may play a role among gastric protective mechanisms by its ability to maintain microvascular integrity (Whittle, 1993), which is also known to be an important factor in the development of mucosal injury (Robert and Kauffman, 1989). In the case of indomethacin-induced gastric mucosal injury, this NO-mediated microcirculatory mechanism is more likely to be involved, since in this model, vascular factors are known to have crucial pathogenic role (Robert and Kauffman, 1989; Whittle, 1993). Indeed, in a time-response study, we found that microvascular injury preceded mucosal damage following indomethacin administration, while the opposite could be observed with cysteamine (our unpublished results). In our present study, we found that in ovariectomized rats the mucus secretion in the stomach has been increased, an effect reversed by L-NAME. Thus, NO mediates mucus overproduction in the ovariectomized rat. This mucus hypersecretion may be the common pathway, which explains why the estrogen-deficient state protects the gastric mucosa against different ulcerogenic stimuli.

In conclusion, estrogen-deficiency provokes a NO-mediated gastric mucus hypersecretion, which improves mucosal defence.

Acknowledgements

Ferenc László was supported by the Széchenyi Professor Fellowship of the Hungarian Ministry of Education.

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