Mechanisms Involved in Aggravation of Ethanol-Induced Gastric Mucosal Lesions in Adrenalectomized Rats

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Abstract—Effects of adrenalectomy (AD) on ethanol-induced gastric injury and prostaglandin (PG) protection on the damage were investigated in rats and compared with those of N-ethylmaleimide (NEM), a sulfhydryl (SH) blocker, and diethyl maleate (DEM), a SH depletor. Oral administration of 100% ethanol (1 ml) induced elongated bands of hemorrhagic lesions in the corpus mucosa of sham operated rats, and these lesions were significantly prevented by 16,16-dimethyl PGE₂ (dmPGE₂, 10 μ g/kg, s.c.). AD markedly enhanced the mucosal ulcerogenic responses caused by ethanol and abolished the protective effect of dmPGE₂; this agent rather worsened the lesions, which appeared throughout the corpus mucosa. AD by itself enhanced the microvascular permeability in the gastric mucosa without any effect on SH contents. These alterations caused by AD were significantly reverted by hydrocortisone treatment (10 mg/kg/day for 2 weeks, s.c.). On the other hand, a single injection of NEM (10 mg/kg, s.c.) similarly enhanced the vascular permeability, worsened the ethanol-induced lesion, and mitigated the protective effect of dmPGE₂ without altering mucosal SH contents, while DEM (1 ml/kg, s.c.) significantly reduced the mucosal SH levels and the lesions. These results suggest that AD worsened the mucosal lesions induced by ethanol, probably by enhancing the microvascular permeability, and this action may be due to a lack of steroid secretion but is not directly related to a mucosal SH deficiency.

Recent studies have shown that the adrenal glands modified the pathogenesis of gastric mucosal lesions in a variety of experimental ulcer models. Hernandez et al. (1) showed that adrenalectomy reduced the protective action of neurotensin against stress-induced gastric lesions in rats. Szabo et al. (2) also reported a significant attenuation of the mucosal protective action of prostaglandin (PG) F_{2 β} against gastric damage induced by ethanol. On the other hand, glucocorticoids protected the mucosa from ethanol insult (3) and restored the mucosal protective effect of PGF_{2 β} (2). However, the mechanism for how adrenalectomy produced such alterations in the mucosal ulcerogenic responses remains unknown.

Szabo et al. (4) and Pihan et al. (5) demonstrated a significant prevention by 16,16-dimethyl PGE₂ (dmPGE₂) and a sulfhydryl (SH) drug against the increased vascular permeability and gastric lesions caused by ethanol; and they proposed that endogenous SHs and vascular endothelium may be considered as a protector and a target, respectively, in the pathogenesis of acute gastric mucosal injury.

The present study was therefore designed to investigate factors responsible for pathogenetic modification caused by adrenalectomy in rats using ethanol-induced gastric lesions and to compare the effects of adrenalectomy with those of N-ethylmaleimide (NEM), a SH blocker (6). We discussed the influences of adrenalectomy on the basis of mucosal vascular permeability and endo-

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genous SH contents.

Materials and Methods

Male Sprague Dawley rats (200–230 g) were used in all experiments. Each study was carried out using 6 to 16 rats per group.

General protocols: The experiments were performed in the following three groups: A. sham operation, B. adrenalectomy, C. adrenalectomy plus hydrocortisone treatment. In groups B and C, bilateral adrenalectomy was performed by a translumbar route under ether anesthesia, and the adrenal glands were removed carefully by forceps. The animals were fed on normal chow and 2% NaCl solution after the operation. In group C, the animals received hydrocortisone acetate subcutaneously once daily for 2 weeks (Sigma Chemicals, 10 mg/kg/day) after the operation (2). In group A, bilateral body walls at the lumbar portion were incised, the incisions were closed without removing the adrenal glands, and the animals were fed on normal chow and tap water. The animals in each group were used in the experiments 2 weeks after the operation, and they were deprived of food for 18 hr prior to the experiments. In these three groups, the mucosal ulcerogenic response to ethanol, mucosal permeability, and mucosal sulfhydryl (SH) contents were measured. In a parallel study, the effects of N-ethylmaleimide (NEM), a SH blocker, were examined on the above parameters and compared with those of adrenalectomy.

Induction of gastric mucosal injury by ethanol: The animals were given 1 ml of absolute ethanol orally by esophageal intubation, and they were killed 1 hr later. The stomachs were removed, inflated by injecting 10 ml of 2% formalin, immersed in 2% formalin for 10 min to fix the gastric wall, and opened along the greater curvature. The area (mm²) of the reddish part of the mucosa was measured under a dissecting microscope with a square grid ($\times 10$), and the lesion index was expressed as the percentage (%) of such areas to the total glandular mucosal area. 16, 16-Dimethyl prostaglandin E₂ (dmPGE₂; Funakoshi, 10 μ g/kg) was given subcutaneously 30 min before administration of ethanol. NEM (Sigma, 10 mg/kg) or diethyl maleate (DEM; Sigma, 1 ml/kg), a SH

depletor, was given subcutaneously 1 hr before ethanol treatment. In each study, the person measuring the lesions did not know the treatment given to the animals.

Measurement of mucosal vascular permeability: The mucosal vascular permeability was determined using a dye according to a previous study (7). In the animals subjected to sham operation, adrenalectomy, adrenalectomy plus hydrocortisone, and treatment with NEM, DEM or dmPGE₂, 1 ml of Evans blue (Sigma, 1% w/w) was injected intravenously, and they were killed 30 min later. NEM, DEM or dmPGE₂ was given subcutaneously 1 hr before sacrifice of the animals. Under ether anesthesia, the animals were killed by bleeding from the descending aorta, the stomachs were removed, and the amount of dye trapped for 30 min in the corpus mucosa and in the gastric contents was measured. After collecting the gastric contents carefully by lavaging with 5 ml of distilled water, the stomach was opened along the greater curvature and the corpus mucosa was scraped off using two glass slides, weighed and put into a tube containing 5 ml of distilled water. The extraction of dye was performed according to the method described by Katayama et al. (8), and the absorbance of samples was measured at 620 nm in a Hitachi spectrophotometer (Model 200-100). The amount of dye recovered from the gastric contents and the corpus mucosa was expressed as micrograms per milliliter of gastric juice and micrograms per 100 mg of tissue, respectively.

Measurement of mucosal sulfhydryl contents: The amount of non-protein SH was measured in the gastric mucosa of rats following adrenalectomy with or without hydrocortisone treatment, and administration of NEM, DEM or dmPGE₂, according to the modified method described by Kaplowitz et al. (9). The animals were killed 2 weeks after adrenalectomy or 2 hr after treatment with the above drugs. The stomachs were removed and incised along the greater curvature, and the corpus mucosa was scraped off using two glass slides and kept cold on ice. The mucosal scrapings were weighed, homogenized in 2 ml of phosphate buffer (0.1 N NaPO₄ plus 0.25 N sucrose, pH 7.4), and centrifuged at

4000 r.p.m. for 15 min at 4°C. Then, 0.5 ml of 25% trichloroacetic acid was added to 1 ml of the supernatant of each sample and allowed to stand for 30 min. After centrifugation for 15 min at 3000 r.p.m., the supernatant was used to determine SH using DNTB (5-5'-dithio-2-nitrobenzoic acid, Wako). Absorbance was measured at 412 nm in a Hitachi spectrophotometer, and the results were expressed as micromoles per g wet tissue weight.

Preparation of drugs: Both NEM and DEM were dissolved in saline, while dmPGE₂ was first dissolved in absolute ethanol and diluted

with saline to the desired concentration. Hydrocortisone acetate was suspended in saline with a trace of Tween 80 (Nakarai). Each drug was prepared immediately before use and given in a volume of 0.5 ml per 100 g of body weight. Control animals received saline in the same volume.

Statistics: Data are presented as the mean $\pm$ S.E. from 6 to 16 rats per group. Statistical analysis was performed using a two tailed Dunnett's multiple comparison test (10), and values of $P < 0.05$ were regarded as significant.

Fig. 1. Gross appearance of gastric mucosal lesions induced by ethanol in rats. Animals were given 1 ml of 100% ethanol orally by esophageal intubation, and they were killed 1 hr later. DMPGE₂ (10 µg/kg) was given subcutaneously 30 min before ethanol treatment. Figures show: A, sham operated rat; B, adrenalectomized rat; C, dmPGE₂-treated adrenalectomized rat. Note that adrenalectomy not only worsened ethanol-induced lesions but also abolished the protective effect of dmPGE₂ on these lesions.

Results

Influences of adrenalectomy on ethanol-induced gastric mucosal lesions: Oral administration of absolute ethanol in sham operated rats caused multiple elongated bands of hemorrhagic lesions in the corpus mucosa within 1 hr (Fig. 1A), the lesion index being $10.8 \pm 2.1\%$ of the mucosa. Gastric lesions induced by ethanol were markedly worsened in the animals subjected to bilateral

adrenalectomy, and the lesion index reached about 3 times greater than the control (Figs. 1 and 2A). Of interest, while subcutaneously administered dmPGE_2 ($10 \mu\text{g/kg}$) almost completely prevented the formation of gastric lesions in response to ethanol in sham operated rats, this agent failed to afford mucosal protection in adrenalectomized rats and rather worsened these lesions. In these animals treated with dmPGE_2 , the injury was not localized as band-like lesions but was ob-

Fig. 2. Effects of adrenalectomy on ethanol-induced gastric lesions and the protective effect of dmPGE_2 against these lesions (A) and on the mucosal vascular permeability (B) in rats. Hydrocortisone (10 mg/kg/day) was given subcutaneously once daily for 14 days after adrenalectomy. In Fig. A, ethanol ($1 \text{ ml, } 100\%$) was given orally by esophageal intubation, and dmPGE_2 ($10 \mu\text{g/kg}$) was given subcutaneously 30 min before ethanol treatment. Data are presented as the mean $\pm$ S.E. from 8 to 16 rats per group. *, * Statistically significant difference from the corresponding controls, at $P < 0.05$.

served as more generalized lesions (see Fig. 1C). The worsening effect of adrenalectomy on ethanol-induced gastric lesions was almost totally attenuated by treatment of these animals with hydrocortisone (10 mg/kg/day for 2 weeks), and in these animals, dmPGE₂ again showed a potent mucosal protection against ethanol.

Effects of sulphydryl blocker and depletor on ethanol-induced gastric mucosal lesions: In normal rats, gastric lesions induced by ethanol were significantly inhibited by both dmPGE₂ (10 µg/kg) and DEM (1 ml/kg), but

markedly aggravated by pretreatment with NEM (10 mg/kg) (Fig. 3A). Gastric lesions induced by ethanol in the NEM-treated animals appeared in wide bands of lesions along the long axis of the stomach, similar to those observed in adrenalectomized rats. Of interest, pretreatment of the animals with NEM completely abolished the protective activity of dmPGE₂, and the lesions rather significantly worsened as compared to the control group.

Effects of adrenalectomy and NEM on gastric mucosal vascular permeability: The

Fig. 3. Effects of dmPGE₂ (10 µg/kg), NEM (10 mg/kg) and DEM (1 ml/kg) on ethanol-induced gastric mucosal lesions (A) and on the vascular permeability (B) in rats. In Fig. A, ethanol (1 ml, 100%) was given orally by esophageal intubation; and NEM, DEM or dmPGE₂ was given subcutaneously 1 hr or 30 min, respectively, before ethanol treatment. In the combined treatment of dmPGE₂ plus NEM, the former was given 30 min before administration of NEM. Data are presented as the mean ± S.E. from 6 to 9 rats per group. *Statistically significant difference from the controls, at $P < 0.05$.

Fig. 4. Effects of adrenalectomy and various agents on gastric mucosal sulfhydryl (SH) levels in rats. dmPGE₂ (10 μg/kg), NEM (10 mg/kg) or DEM (1 ml/kg) was given subcutaneously, and hydrocortisone (10 mg/kg/day) was given subcutaneously once daily for 14 days after adrenalectomy. The animals were killed 2 hr after administration of each drug. Data are presented as the mean±S.E. from 6 to 8 rats per group. *Statistically significant difference from the controls, at $P < 0.05$.

vascular permeability of the gastric mucosa under normal conditions was minimal when determined as an accumulation of Evans blue in both the gastric mucosa and contents, the values being 0.5–1.7 μg/ml of gastric juice and 3–4 μg/100 mg tissue (Figs. 2B and 3B). Bilateral adrenalectomy significantly increased the mucosal vascular permeability, and the amount of dye in the gastric contents was increased to about 5-fold greater than the control values. The increased vascular permeability caused by adrenalectomy was significantly suppressed by treatment with hydrocortisone, and the values were not statistically significant from those observed in normal rats. Neither dmPGE₂ nor DEM had any effect on the mucosal vascular permeability, but NEM did significantly augment the amount of dye accumulated in both gastric mucosa and contents.

Effect of adrenalectomy and NEM on mucosal SH contents: The levels of mucosal SH (non-protein sulfhydryls) in normal rat stomachs were 2.54 ± 0.32 μmol/g tissue, and

they remained in the same range following sham operation or bilateral adrenalectomy (Fig. 4). Treatment of the animals with either dmPGE₂, NEM or hydrocortisone did not significantly affect the SH contents in the gastric mucosa, while administration of DEM significantly reduced the levels of mucosal SH to 1/3 of the normal values.

Discussion

The present study confirmed the finding by Szabo et al. (2), who showed that adrenalectomy in rats inhibits the usual protective effect of a PG on ethanol-induced injury of the gastric mucosa, and the results further suggested a possible involvement of the increased vascular permeability caused by adrenalectomy in this phenomenon.

A relationship between the adrenal glands and gastric lesions has been postulated in experimental animals using stress ulcers, indomethacin-induced lesions and ethanol-induced gastric lesions (1, 2, 11). Most of the studies showed aggravation of the lesions

following adrenalectomy, although the mechanism remains unknown. Szabo et al. (2) demonstrated that adrenalectomy attenuated the usual protective effect of $\text{PGF}_{2\beta}$ and SH drugs or cimetidine on ethanol- or aspirin-induced gastric lesions. In agreement with those data, the present study showed that adrenalectomy by itself worsened ethanol-induced gastric lesions but also attenuated the protective effect of dmPGE_2 against these lesions. These findings suggest that adrenalectomy produces a generalized worsening effect, irrespective of the types of lesions or the types of antiulcer drugs. Since the medullectomy had no effect on indomethacin-induced gastric lesions (11) and rather inhibited ethanol-induced gastric injury (2), it seems likely that the worsening effect of adrenalectomy is due to removal of the adrenal cortex. This notion is supported by the present finding that replacement with hydrocortisone significantly prevented the pathogenetic modification of ethanol injury in the adrenalectomized animals.

Ethanol-induced gastric lesions appeared usually as elongated bands of damage along the long axis of the stomach. Adrenalectomy induced more wide bands of the lesions in response to ethanol, and it caused more generalized lesions throughout the corpus mucosa in the presence of dmPGE_2 . Recent studies indicate that microvascular alterations may play a critical role in acute gastric mucosal damages. Intra-gastric administration of ethanol also results in rapidly developing vascular injury in subepithelial capillaries and increases the vascular permeability as measured by Evans blue extravasation (4, 12). Of interest, adrenalectomy significantly increased the amount of extravasated dye in the mucosa, suggesting a possible involvement of enhanced vascular permeability in the mechanism through which ethanol-induced lesions worsened. Since dmPGE_2 alone slightly increased the vascular permeability and is known to potentiate the permeability responses caused by other agents (6, 13), the permeability alterations may be associated with the pathogenetic modification observed in the adrenalectomized rats. This contention is supported by the findings that NEM also increased the vascular permeability, worsened

the ethanol-induced lesions, and attenuated the protective effect of dmPGE_2 . In addition, glucocorticoid treatment significantly reversed the enhanced vascular permeability and mucosal ulcerogenic responses caused by adrenalectomy. Since glucocorticoids are known to induce endogenous factors (vasoregulin) which inhibit the vascular permeability (14), a lack of steroid secretion induces the vascular fragility, the increased vascular permeability, and thereby enhances the mucosal ulcerogenic response caused by ethanol.

On the other hand, it has been suggested that mucosal SH is considered to be a protector against gastric damage induced by necrotizing agents (15). Thus, it is possible to assume that adrenalectomy modified ethanol-induced mucosal lesions by means of reducing endogenous SH levels. However, the present study excludes the above possibility for the following reasons: (a) both adrenalectomy and NEM significantly worsened ethanol-induced gastric lesions without altering the mucosal SH levels. (b) DEM, a SH depletor, significantly reduced SH contents in the gastric mucosa, yet inhibited the mucosal lesions induced by ethanol. We found that the functional perturbations of endogenous SH by NEM markedly enhanced the microvascular permeability responses and mucosal lesions induced by ethanol. Especially, this agent significantly augmented the microvascular permeability in normal rats without exposure to ethanol, suggesting that alkylation of SH makes the vasculature fragile to adversely affect the permeability of endothelial or parenchymal cells. Since recent studies showed that SHs are involved in nonreceptor modulation of contractile activity of the vascular smooth muscle (16, 17), the increased vascular permeability caused by NEM may be in part attributed to functional disturbances of such nonreceptor systems responsible for maintenance of normal vascular integrity. Although NEM did not alter the mucosal SH levels, endogenous SH may be functionally linked to the regulation of the microvascular permeability and thereby is important in protecting the gastric mucosa against damage. It may be possible that adrenalectomy, similar to NEM, influences

such SH dependent systems without reducing endogenous SH levels and enhances the mucosal vascular permeability and lesions.

It is intriguing that dmPGE₂ rather worsened ethanol-induced gastric lesions in rats following adrenalectomy or administration of NEM. We have previously shown that the phenomenon of cytoprotection induced by a variety of drugs including dmPGE₂ is accompanied by inhibition of gastric motility and proposed that relaxation of the stomach smooth muscle may be involved in the mechanism of mucosal protection against necrotizing agents (18–21). Inhibition of the stomach contraction would change the necrotizing agents from a "strong irritant" to a "mild irritant" through flattening of the mucosal foldings, and it reduces the severity of macroscopically visible lesions induced by necrotizing agents. However, both adrenalectomy and NEM may induce vascular fragility and sensitize the vasculature to irritants. Under these situations, even though dmPGE₂ by inhibiting the stomach contraction dissolved the mucosal foldings and reduced the irritative activity of ethanol, it seemed noxious enough to induce hemorrhages in the mucosa. This speculation would be supported by the finding that the morphology of the damage was changed from elongated bands into more generalized lesions in the presence of dmPGE₂ (in the absence of mucosal folds).

Taken together, the results of the present study suggested that adrenalectomy worsened the mucosal ulcerogenic responses caused by ethanol and abolished the protective effect of dmPGE₂, probably by enhancing the microvascular permeability in the gastric mucosa. These effects may be due to a lack of steroid secretion caused by adrenalectomy, but not related to a mucosal SH deficiency.

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