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ANTIULCER ACTIVITY OF HYPERTONIC SOLUTIONS IN THE RAT: POSSIBLE ROLE OF PROSTAGLANDINS

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The effects of hypertonic solutions on gastric acidity and on experimental gastric and duodenal ulcers as well as on gastric prostaglandins (PGs) were studied in the rat. The oral administration of a 10% NaCl solution resulted in complete absence of free acidity and very significant reductions in total acidity 24 h after pyloric ligation. The antiulcer effect of hypertonic saline was studied in three experimental models. In pyloric-ligated rats, both the incidence and the severity of gastric ulcers were remarkably reduced by hypertonic saline treatment. Indomethacin-induced gastric erosions were significantly reduced by hypertonic NaCl or sorbitol and completely prevented by hypertonic xylitol. Cysteamine-induced duodenal ulcers were also significantly reduced by hypertonic solutions of NaCl, xylitol or sorbitol. In the latter model, indomethacin potentiated the ulcerogenic effect of cysteamine and also reduced the efficacy of the hypertonic NaCl gavage. The possible contribution of PGs to these effects was further investigated by analysing PGE in the gastric mucosa and juice. Rats treated orally with hypertonic NaCl solutions had several-fold higher PGE contents in their gastric mucosa as well as higher PGE levels in the gastric juice. It is concluded that hypertonic solutions stimulate endogenous PGE biosynthesis and also exert profound antiulcer effects in the rat. A causal relationship between the two phenomena is suggested.

Ulcer Gastric acidity Prostaglandins Hypertonic solutions

1. Introduction

We have recently reported that in whole cell preparations in vitro, hypertonicity of the buffer was a powerful stimulant of prostaglandin (PG) biosynthesis in the rat stomach (Assouline et al., 1977) as well as in the rat renal medulla (Danon et al., 1978). If the same is true in vivo, namely if hyperosmolarity of the stomach contents could enhance PG biosynthesis in situ, then one would expect that increased osmolarity would reproduce some of the known biological effects of PGs on the stomach. One could predict that increasing the osmotic concentration of the stomach contents would result in inhibition of acid secretion and prevention of experimental ulcers. Indeed, earlier investigators have established the fact that hypertonic solutions of sodium chloride or glucose given either orally (Cataland et al., 1974), intraduodenally (Matsuyama, 1932; Sircus, 1958), or intravenously (Baume et al., 1965; Thorsoe, 1971) all inhibited gastric acid secretion in experimental animals and in humans. The present investigation was therefore undertaken to assess the in vivo effects of intragastric hypertonic solutions on gastric acidity and peptic ulceration vis-à-vis the possible contribution of increased PG biosynthesis to these effects. Part of this work has been presented in an abstract (Assouline and Danon, 1978).

2. Materials and methods

2.1. General

All experiments were carried out on female Charles River rats weighing 150-200 g. Animals were randomly assigned to treatment or control groups and all observations were made by two independent workers in a blind fashion.

2.2. Pyloric-ligated rats

Pyloric ligation was carried out on 24 h-fasted rats under light ether anesthesia. 2 ml of either hypertonic (3400 mOs/l) or normal (300 mOs/l) saline were administered by stomach tube initially and again at 6 h. The rats were sacrificed at 24 h, at which time the volume of the gastric contents was noted and the pH, acidity and PGE concentration were measured. Stomachs that had been perforated (2 out of 11 in the isotonic-treated group) could not be used in this analysis. Comparisons between groups were carried out using Student's *t*-test. The mucosa of the stomach was examined for the presence of ulcers. Preliminary observations indicated a higher incidence and greater reproducibility of ulcers in the secretory part of the stomach rather than in the forestomach as described by Shay et al. (1945). This may represent a strain or an environmental variation. The number of ulcers was noted for each rat and the severity was evaluated from that of the most acute lesion, on a scale of 0 to 3+. The "ulcer index" was calculated according to Robert et al. (1968) as the sum of (a) percent incidence (divided by 10) of animals with ulcers, (b) average severity of ulcers for each group, and (c) average number of ulcers per rat for each group. For statistical analysis, each animal was given a rating composed of the sum of number of ulcers and their severity. Ratings of individual rats were ranked in order of size, and comparisons between groups were made using the rank sum test (Wilcoxon). In one experiment, rats were also treated with indomethacin (Sigma, St. Louis, Mo.). Indomethacin was freshly suspended in water with Tween 80 at a concentration of 6 mg/ml and injected s.c. 15 mg/kg initially, followed by 10 mg/kg at 12 h. Control rats were treated with the vehicle.

2.3. Indomethacin-induced gastric erosions

Rats were fasted for 24 h, following which indomethacin was injected i.p. 20 mg/kg. Indomethacin solutions were freshly prepared (8 mg/ml) in 2% sodium bicarbonate, pH < 8. 2 ml of hypertonic solutions of either NaCl (analytical), xylitol (Sigma, St. Louis, Mo.) or sorbitol (BDH, Poole, England) or normal saline (300 mOs/l) in controls were given by stomach tube 30 min before indomethacin. The rats were sacrificed 5 h after the injection of indomethacin and the stomachs were examined for mucosal erosions. Due to the peculiar nature of the lesions produced in this model, ulcers were not counted as in the other models, but were evaluated only in terms of severity, on a scale of 0 to 4+. The "ulcer index" was calculated accordingly from the percent incidence and average severity of the erosions.

2.4. Cysteamine-induced duodenal ulcers

These were produced and evaluated according to Robert et al. (1974). Cysteamine hydrochloride (Sigma, St. Louis, Mo.), 425 mg/kg, was injected s.c. to rats fed ad libitum on laboratory chow. 2 ml of a hypertonic solution, or normal saline in controls, were injected initially and at 6 h. In some experiments, indomethacin (suspended in water by addition of Tween 80) was additionally injected s.c. 15 mg/kg initially, followed by 10 mg/kg at 12 h. All rats were sacrificed at 24 h and their duodenums examined. Calculation of the "ulcer index" and statistical analysis were carried out as described for the pyloric-ligated rats.

2.5. PG determination

PGE-like activity was analysed by radioimmunoassay utilizing antiserum to PGE-BSA (Miles-Yeda Ltd., Rehovot, Israel, lot no. PRE2). (^{3}H)-PGE was purchased from the Radiochemical Center, Amersham (lot no. 18), specific activity 160 Ci/mmol. The radioimmunoassay responded equally to PGE_1 and PGE_2 and also cross-reacted with PGAs and PGBs. Cross reaction with $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ was less than 3 and 4%, respectively. PGE in the gastric juice of pyloric-ligated rats was determined without extraction, as gastric juice did not interfere with the assay. On the other hand, PGE in homogenates of gastric mucosa from cysteamine-treated rats was analysed after extraction as follows: the rats were sacrificed 2 h after cysteamine treatment and gastric gavage, the stomach rinsed with tap water and the mucosa immediately scraped with a glass slide, weighed and homogenized in chloroform : methanol (2 : 1, v/v). The extract was dried under nitrogen, redissolved in 2 ml of diethyl ether and extracted into 10 ml of phosphate buffer, pH 7. The PGs were finally reextracted into 10 ml ether after acidification of the aqueous phase with 0.1 N HCl to pH 2-3. The ether layer was then evaporated and the PGE dissolved in phosphate buffer. Recovery of labeled PGE which was extracted in parallel was 80%.

2.6. Analysis of gastric acidity

Free and total acidity were titrated with 0.1 N NaOH, using Topfer's reagent and phenolphthalein as indicators.

3. Results

3.1. Effect on gastric acidity

Hypertonic saline caused a significant reduction in acid concentration and content of the ligated stomachs. Free acidity did not recover after treatment with hypertonic (3,400 mOs/l) saline (0.0 in a group of 10 rats, compared with a mean ± S.E.M. of 21.0 ± 2.8 mEq/l in 9 control rats, $P < 0.001$), while total acid concentration and content were reduced from 39.0 ± 1.3 to 13.0 ± 3.2 mEq/l ($P < 0.001$) and from 0.40 ± 0.05 to 0.22 ± 0.05 mEq ($P < 0.05$), respectively. In the same rats, the pH of the gastric juice increased from 2.1 ± 0.16 to 6.5 ± 0.82 ($P < 0.001$), and its volume from 10.1 ± 0.9 to 17.3 ± 1.8 ml ($P < 0.005$).

3.2. Antiulcer effect

3.2.1. Pyloric ligation-induced ulcers

In pyloric-ligated rats, the hypertonic sodium chloride solution was highly protective against gastric ulcers. Both the mean number of ulcers per rat and their average severity were greatly reduced, resulting in an ulcer index of 2.8 in 10 rats, compared with 13.3 in 11 controls ($P < 0.01$). It may be of interest to note that gross hyperemia of the stomach was observed in all the hypertonic-treated rats.

3.2.2. Indomethacin-induced gastric erosions

The characteristic gastric erosions produced 5 h following the injection of indomethacin were inhibited after treatment with xylitol, 3000 mOs/l (ulcer index 0, ($n = 7$) as compared with 12.1 in 8 rats receiving normal saline, $P < 0.01$) and greatly reduced after the instillation of hypertonic sodium chloride (ulcer index of 4.0 in 7 rats, $P < 0.01$) or sorbitol (ulcer index of 3.4 in 8 rats, $P < 0.01$) at the same osmolarities. Lower concentrations of xylitol, down to 1000 mOs/l, were tested and also found to be significantly protective (fig. 1). Also, a marked accumulation of fluid was noted on gross examination of the gut in rats that had received xylitol in osmolarities higher than 2250 mOs/l. However, fluid accumulation was minimal with 1000 mOs/l, while protection against ulcers was still significant.

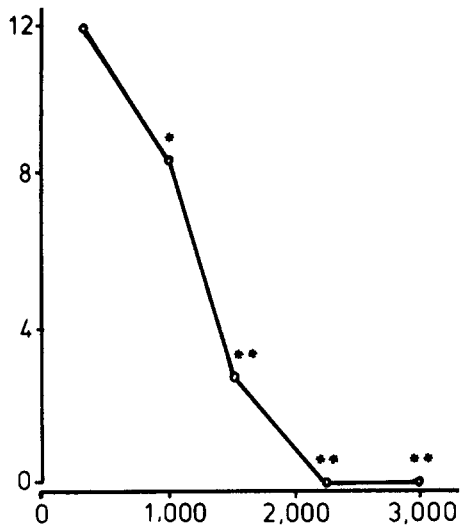

Fig. 1. Protection against indomethacin-induced gastric erosions by varying osmolarities of xylitol. Each point represents the ulcer index derived from 7 rats. * $P < 0.05$, ** $P < 0.01$, compared with rats given an isotonic solution. *Ordinate:* ulcer index. *Abscissa:* xylitol (mOs/l).

3.2.3. Cysteamine-induced duodenal ulcers

The protective effects of hypertonic solutions on gastrointestinal ulceration were finally corroborated in this experimental model. Table 1 shows the ulcer indices in hypertonic saline-treated and in control rats, as well as in groups of rats additionally treated with indomethacin (15 + 10 mg/kg). The results indicate complete inhibition of the formation of duodenal ulcers by gavage with hypertonic NaCl. Also, while indomethacin potentiated the ulcerogenic effect of cysteamine, hypertonic saline also provided highly significant protection against the combined effect of cysteamine and indomethacin. In another experiment, hypertonicity was produced with either NaCl, xylitol or sorbitol. As shown in table 1, significant protection against ulcers was observed with both sugars as well as with saline at 3000 mOs/l each.

It may also be of interest that 16 out of a

TABLE 1

Effects of hypertonic solutions and of indomethacin (15 mg/kg initially followed by 10 mg/kg at 12 h) on cysteamine-induced duodenal ulcers.

Group No.	Treatment	n	Ulcers			
			Incidence %	Severity	Number/rat	Index
Experiment I						
a	Isotonic control	9	89	1.6	1.3	11.8
b	Hypertonic NaCl (3000 mOs/l)	8	12	0.1	0.1	1.5 ($P < 0.01$) [1]
c	Hypertonic xylitol (3000 mOs/l)	8	12	0.1	0.1	1.4 ($P < 0.01$) [1]
d	Hypertonic sorbitol (3000 mOs/l)	6	33	0.2	0.3	3.8 ($P < 0.01$) [1]
Experiment II						
e	Isotonic control	10	70	0.4	1.1	8.5
f	Hypertonic NaCl (3400 mOs/l)	8	0	0	0	0 ($P < 0.01$) [2,3]
g	Isotonic + indomethacin	7	100	1.9	1.9	13.8 ($P < 0.01$) [2,3]
h	Hypertonic NaCl + indomethacin	9	44	0.6	0.5	5.5

[1] Significant difference compared with isotonic control (group a).
[2] Significant difference compared with isotonic control (group e).
[3] Significant difference compared with group h.

total of 38 rats that had been treated with the combination of cysteamine and any of the hypertonic solutions died before the end of 24 h. Therefore, they were not included in the calculation of the "ulcer index". This introduced only a negative bias, if at all, since at autopsy the rats that had died failed to exhibit any duodenal ulceration. Similar toxicity has been reported for the combination of 16,16-dimethyl-PGE_2 and cysteamine (Robert et al., 1974). Although the mechanism of this lethal interaction remains unclear, it may depend on the presence of large quantities of PGs, either endogenous or exogenous. This conclusion is supported by the fact that indomethacin, while potentiating the ulcerogenic effect of cysteamine, reduced the lethality of its combination with hypertonic saline (only 1/10 died).

3.3. Effect on gastric PGE

Table 2 shows that hypertonic NaCl caused significant augmentations in PGE concentration and content in the gastric juice whether the rats were treated with indomethacin (7-fold increase in content) or not (5-fold increase). In addition, the data indicate that indomethacin caused significant reduction in PGE concentration and content in the hypertonic-treated rats as well as in the controls.

The PGE content of the gastric mucosa of cysteamine-treated rats was determined in groups of 6 rats each, 2 h following gavage. The mean (± S.E.M.) mucosal content of PGE was 13.7 ± 2.2 ng/100 mg wet tissue in rats treated with 2 ml of a 10% solution of NaCl, compared with 4.6 ± 0.7 in the rats that had received isotonic saline ($P < 0.005$). This was a 3-fold increase in mucosal PGE content of the treated rats as compared with the controls.

4. Discussion

The data show that the intragastric administration of hypertonic solutions of varied natures causes a drastic reduction in acid concentration and content and also protects against gastric and duodenal ulcers in the three experimental models tried in the rat. Moreover, we have presented evidence for the concomitant enhancement of endogenous PGE production. In cysteamine-treated rats, the PG content of the mucosal homogenate increased several-fold after hypertonic treatment. These concentrations may represent actual tissue levels of PGs at the moment of death or the capacity of the mucosa exposed

TABLE 2

PGE in the gastric juice of pyloric-ligated rats: effect of hypertonic NaCl and indomethacin (15 mg/kg) initially followed by 10 mg/kg at 12 h. Rats were sacrificed at 24 h. Mean ± S.E.M. (number of rats per group indicated in parentheses).

Treatment	NaCl 0.9% (9)	NaCl 10% (10)	Indomethacin + NaCl 0.9% (4)	Indomethacin + NaCl 10% (14)
PGE concentration (ng/ml)	0.28 ± 0.03	0.79 ± 0.12 ($P < 0.001$) [1]	0.17 ± 0.04 ($P < 0.05$) [2]	0.42 ± 0.06 ($P < 0.005$) [1] ($P < 0.02$) [2]
PGE content (ng/stomach)	2.71 ± 0.32	12.9 ± 1.60 ($P < 0.001$) [1]	1.21 ± 0.42 ($P < 0.02$) [2]	8.03 ± 1.07 ($P < 0.001$) [1] ($P < 0.02$) [2]

[1] Significant difference compared with isotonic-treated controls.
[2] Significant difference compared with the related non-indomethacin-treated controls.

to hypertonicity in vivo to elaborate PGs during workup (Piper and Vane, 1971), after washout of the hypertonic environment. In any event, there appears to be an increased biosynthetic capacity of the tissue that had been exposed to hypertonicity. This is also supported by our previous report that in a whole cell preparation of the rat stomach, PG biosynthesis was highly stimulated in hypertonic media (Assouline et al., 1977). Likewise, in the pyloric-ligated rats, where PGE was determined in the gastric juice, the amount of PGs released into the stomach lumen was significantly elevated in the hypertonic saline-treated rats. Although the possible contribution of a pH-partition effect to the accumulation of PGs in the gastric juice of the treated rats cannot be ruled out, these observations support our conclusion that hypertonic solutions do enhance PGE synthesis in the rat stomach.

PGE, and, more recently, prostacyclin (PGI_2), have been shown to be produced in the stomach and also to exert some fundamental effects on this organ (Moncada et al., 1978; Whittle et al., 1978). Both PGE and PGI_2 are powerful inhibitors of acid secretion; they increase mucosal blood flow, exert a cytoprotective effect on the gastrointestinal tract and inhibit ulcer formation in a number of experimental models. The present data show that orally administered hypertonic solutions produce all three effects in the rat. Thus, gastric acidity and ulcer formation were inhibited. Also, there was marked hyperemia of the stomach, which is consistent with increased mucosal blood flow.

Thus, the data tend a suggest a causal relationship between the biological activities effected by the intragastric administration of hypertonic solutions and the increased PGE output that was observed. Indomethacin, a PG-synthetase inhibitor, was used on several occasions in the course of the present study vis-a-vis a prostaglandin-mediated effect of the hypertonic solutions. Indomethacin was used as an ulcerogenic agent, in the light of the suggestion that the lesions induced by this drug may represent a PG deficiency, and therefore can be prevented by exogenous PGs (Lippmann, 1974). Indomethacin was also used in conjunction with cysteamine as well as with pyloric ligation. Although the effects of indomethacin do not provide unequivocal evidence for the mediation of PGs in the antiulcer activity of hypertonic solutions, they are clearly compatible with such a theory. At the doses used, indomethacin partly inhibited but did not abolish gastric PG synthesis (table 2) while hypertonic solutions caused increased output of PGs. Consequently, the net effect was the result of the interplay between these two factors. Thus, hypertonic solutions increased the rate of PG synthesis that had been diminished by indomethacin, and prevented ulcers. Viewed differently, indomethacin attenuated the effect of hypertonic solutions in the cysteamine model and reduced their protective effect.

Other mechanisms for the effects of hypertonic solutions on the stomach may also be considered. An enteric mechanism (Sircus, 1958; Cataland et al., 1974) seems unlikely in view of the fact that some of our experiments were carried out in the pyloric-ligated rat. Alternatively, the reduced gastric acidity that was observed could be explained by the possible breakage of the mucosal barrier by the hypertonic solution, with resultant acid back-diffusion. The enhanced production of PGE could thus be secondary to tissue damage. However, such disruption of the mucosal barrier could hardly explain the unmistakable antiulcer effects that were recorded following the instillation of the hypertonic solutions. On the contrary, increased back-diffusion of acid into the gastric mucosa, as is assumed to occur with aspirin, could be expected to cause mucosal damage, not protection (Davenport, 1964).

Little information is available concerning the mechanisms which regulate the production of PGs in the stomach. Vane suggested that the mechanical interaction between the stomach and its contents which accompanies

gastric contractions might stimulated PG synthesis in the mucosa (Vane, 1971). The present data suggest that the osmolarity of the stomach contents may also contribute to the regulation of gastric PG biosynthesis. Indeed, the gastric mucosa is one of the few organs which are affected by markedly varying osmolarities under physiological conditions. Although most of the present experiments were carried out using rather high solute concentrations, the graded concentration-response relationschip (fig. 1) indicates that the antiulcer effect can be elicited at lower solute concentrations. The relevance of these findings to human pathophysiology and possibly to therapy remains to be established.

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