ELECTROPHYSIOLOGICAL APPROACH TO THE ACTION OF TAURINE ON RAT GASTRIC MUCOSA

TOSHIKIRO KIMURA, SHINJI YAMASHITA, KYUNG SOON KIM, AND HITOSHI SEZAKI

Faculty of Pharmaceutical Sciences, Kyoto University, Yosida Shimoadachi-cho, Sakyo-ku, Kyoto 606, Japan

(Received February 5, 1982)

The effect of taurine on isolated rat gastric mucosa was examined electrophysiologically in order to clarify the mechanism of taurine-induced effect on drug absorption. Although taurine itself had little effect on the electrical parameters, potential difference (PD), short-circuit current (I_{sc}), and tissue electrical resistance (R), of the gastric mucosa, sodium lauryl sulfate, an anionic surfactant, was shown to reduce these parameters remarkably.

On the other hand, when aspirin was present, taurine showed different effects on the gastric mucosa depending on the concentrations of aspirin. Taurine decreased I_{sc} significantly in the presence of aspirin at low concentration (1 mM), which has in itself no influence on the electric parameters. In contrast, aspirin at higher concentration (10 mM) was shown to act as a barrier breaker of the gastric mucosa resulting in a marked decline of PD and I_{sc} . When taurine was added, however, these parameters were reasonably recovered. It seems to be likely that taurine acts as a protector against the aspirin-induced damage in the gastric mucosa.

Keywords — electrophysiological approach; taurine; gastric mucosa; aspirin; potential difference; short-circuit current; tissue electrical resistance; rat; gastric absorption

Our previous report showed the complex action of taurine on the absorption behavior of aspirin in rat stomach. Namely, taurine enhanced the absorption of 1 mM aspirin, on the other hand, the absorption of aspirin in percentage increased in the absence of taurine with increasing concentration of the drug, and at 10 mM aspirin, taurine significantly reduced the absorption in comparison with the control.¹⁾ Aspirin is well known as a breaker of the gastric mucosal barrier,²⁾ and these effects of taurine would be due to some changes in the barrier function of the gastric mucosa.

There are some electrophysiological studies carried out recently according to the short-circuit technique of Ussing³⁾ to analyze the change in the permeability of biological membranes. On the gastric mucosa, Fromm *et al.* investigated the effect of salicylate and its structurally related compounds on ion transport processes in rabbits.⁴⁾ In this study, the effect of tau-

rine on rat gastric mucosa is examined electrophysiologically and its correlation with the change in the gastric absorption of aspirin is discussed.

MATERIALS AND METHODS

Preparation of Gastric Mucosa—Male Wistar albino rats, 220–300 g, were anesthetized by an intraperitoneal injection of sodium pentobarbital. The stomach was removed quickly and the external muscular layer was stripped off carefully with sharp scissors with the serosal surface being immersed in the saline.

Incubation of Isolated Rat Gastric Mucosa—The gastric mucosa thus prepared was preincubated in oxygenated Ringer's solution for 15 min at 37°C and was mounted between two Lucite half chambers⁵⁾ with a 1.13 cm² opening, as shown in Fig. 1. Both sides of the tissue were filled with 11 ml of Ringer's solution, bubbled with 95% O₂–5% CO₂ during the ex-

perimental period. The chamber was placed in the temperature-controlled box. The air temperature was regulated to maintain the solution temperature at 37°C. The composition of the bathing solution in millimoles per liter was as follows: Na, 143.0; K, 5.0; Ca, 1.5; Mg, 1.2; Cl, 153.4; H₂PO₄, 2.2; HCO₃, 24.8; and glucose, 10. The solution was maintained at 37°C and was oxygenated with 95% O₂-5% CO₂ to yield an initial pH of approximately 7.4.

Electrical Measurements—The transmucosal electrical potential differences (PD) was measured *via* a pair of KCl-agar bridges (3% agar in 4 M KCl solution) connected to caromel electrodes which in turn were attached to a high input impedance electrometer, Model DP-506 potentiometer from Kuwano Electric Co., Kawasaki, Japan, and continuously recorded using Model R-111M multirecorder from Shimadzu, Kyoto, Japan. For measurements of the short-circuit current (I_{SC}), the transmucosal PD was maintained at 0 mV by an automatic voltage clamp system.⁶⁾ Another pair of NaCl-agar

bridges (3% agar in 4 M NaCl solution) was used to pass current across the mucosa *via* Ag-AgCl electrodes to measure the I_{SC} . The I_{SC} was measured with Model AD-5513 multimeter from A&D Instrument, Kitamoto, Japan. The tissue electrical resistance (R) was calculated by Ohm's law, taking into account the calculated resistance of the bathing solution. Preliminary experiments indicated that, after mounting the tissue, the PD and I_{SC} reached maximum values at 20 min, followed by slow decrease. The calculated R leveled off to a constant value at 30 min, which

FIG. 1. Schematic Representation of Chambers Used for Determination of Electric Parameters

FIG. 2. Control Values of Potential Difference (PD), Short-circuit Current (I_{SC}), and Tissue Electrical Resistance (R) of Rat Gastric Mucosa at pH 7.4

Each point is the mean of a 5 min measurement ($n=11$) and are plotted at the mid point.

FIG. 3. Effect of Taurine, β -Alanine, and Sodium Lauryl Sulfate (SLS) on Potential Difference (PD; a), Short-circuit Current (I_{sc} ; b), and Tissue Electrical Resistance (R; c) of Rat Gastric Mucosa at pH 7.4

The adjuvants (final concentration, 2 mM) were added to the mucosal solution at 35 min after the mounting of the gastric mucosa: $\bullet$, taurine ($n=6$); $\circ$, β -alanine ($n=4$); $\blacksquare$, SLS ($n=4$). Results are expressed as the relative values to the measured values at 30–34 min, $X(t)/X(30)$, where X is PD, I_{sc} , or R . Dotted lines represent the control values shown in Fig. 2. * $p < 0.001$, compared to the control.

was maintained for following 30 min. Therefore, the experiment was started at 20 min after the mounting and measurements were carried out at 10 min intervals. The I_{sc} was measured by connecting the voltage clamp system for 5–10 s every min and represented by the mean of 5 continuous measurements.

Absorption Experiments—Male Wistar albino rats, 160–200 g, were used. The absorption from the stomach was examined by *in situ* loop methods.⁷⁾ The volume of the drug solution was 4 ml. The composition of the isotonic luminal solution was 0.1N HCl-NaCl, pH 1.1. The amount of drug absorbed in one hour was calculated by the difference in the amount of the drug between the initial and final solutions.

Statistical Analyses—Statistical analyses were performed using Student's t test. The data were expressed as the mean ± 1 SEM.

Materials—Aspirin (Maruishi Pharmaceutical Co., Japan) was used without further purification. Taurine, β -alanine, sodium lauryl sulfate (SLS), and all other reagents used in this study were of the finest grade available from Nakarai Chemical Co., Kyoto, Japan.

RESULTS AND DISCUSSION

Electrical Parameters of Rat Gastric Mucosa

Fig. 2 shows the electrical parameters, PD, I_{sc} , and R , of rat gastric mucosa as a function of time. PD and I_{sc} show monotonical decreasing: PD, 6.7 mV to 3.7 mV; I_{sc} , 70.8 $\mu A/cm^2$ to 40.0 $\mu A/cm^2$ in 40 min. On the other hand, R was maintained at a constant value, 93 $\Omega \cdot cm^2$, during the experimental period. There are no report on the electrical parameters of rat gastric mucosa by the similar technique, but these values resemble ones of rabbit fundus reported by Kivilaakso *et al.*⁸⁾

Effect of Taurine on Electrical Parameters of Rat Gastric Mucosa

Our previous report showed that the gastric absorption of aspirin, at the concentration of 1 mM, was increased by taurine (0.1–10 mM), but not structurally related β -alanine (1 mM), using *in situ* loop technique.¹⁾ In order to elucidate the

FIG. 4. Effect of Taurine on Aspirin-induced Change in Potential Difference (PD; a, b), Short-circuit Current (I_{SC} ; c, d), and Tissue Electrical Resistance (R ; e, f) of Rat Gastric Mucosa at pH 7.4

Aspirin and taurine were added to the mucosal solution at 25 min and 35 min after the mounting of the gastric mucosa, respectively. Final concentration of aspirin was 1 mM (a, c, e) or 10 mM (b, d, f) and that of taurine was 2 mM: $\circ$, aspirin alone ($n=5$ for both concentrations of aspirin); $\bullet$, aspirin + taurine ($n=4$ for both concentrations of aspirin). Results are expressed as the relative values to the measured values at 30–34 min, $X(t)/X(30)$, where X is PD, I_{SC} , or R . Dotted lines represent the control values shown in Fig. 2. ** $p < 0.01$, * $p < 0.05$, compared to the values in the presence of aspirin alone.

action of taurine on the function of the gastric mucosa, the effect of taurine on the electrical parameters of rat gastric mucosa was examined in comparison with those of β -alanine and SLS; the latter, an anionic surfactant, is well known to solubilize biological membranes and increased aspirin absorption to the similar extent of taurine at the concentration range of 0.5–20 mM.⁹⁾ Fig. 3 shows this result. Although the experimental pH in this study is different from one in the absorption experiments, it is difficult to change the pH in this system at present. However, some of the electrophysiological observations given in these experiments would be valid to clarify the action of taurine.

SLS significantly lowered the PD, I_{SC} , and R compared to the controls (dotted lines). This indicates two possibilities: first, the gastric mucosa became leaky by the solubilizing effect of SLS; and second, SLS molecules entered the gastric mucosal cells and damaged the ion transport function. Thus, SLS is shown to have a direct action on the gastric mucosa. SLS-induced increase of aspirin absorption is, however, not explained simply by solubilization of the mucosal barrier, since it depends upon the composition of the luminal medium.⁹⁾ Some of the characteristics will be discussed elsewhere.⁹⁾ On the contrary, neither taurine nor β -alanine change any of the electrical parameters significantly. Although taurine and SLS are shown to act on the gastric mucosa in electrophysiologically dif-

ferent manner, we cannot draw any positive conclusion for the mechanism of action of taurine from this experiment.

Effect of Taurine on Aspirin-Induced Changes in Electrical Parameters

As described above, taurine increased the gastric absorption of 1 mM aspirin. On the other hand, the absorption of aspirin at the high concentration (10 mM), which was significantly higher in percentage than at 1 mM, decreased by the presence of taurine.¹⁾ In this section, the electrophysiological investigation was carried out on these complex actions of taurine, which depend on the concentration of aspirin.

Fig. 4 summarizes the effect of taurine on the electrical parameters of the gastric mucosa in the presence of aspirin. Aspirin and taurine were added to the mucosal solution at 25 min and 35 min after the mounting of the tissue, respectively. As is evident from the figure (a, c, and e), aspirin at the concentration of 1 mM slightly reduced R of the mucosal membrane, but did not affect the PD and I_{SC} at all. However, as shown in Fig. 4(b) and (d), 10 mM aspirin reduced the PD and I_{SC} significantly, compared to each control (dotted lines). The PD has been suggested to be a sensitive index of mucosal integrity.¹⁰⁾ Thus, it is evident that the high concentration of aspirin induced the damage to the gastric mucosa. The effect on R was, however, mild (Fig. 4(f)) and this is the noticeable difference from the effect of SLS, a surfactant (Fig. 3(c)).

TABLE I. *Effect of Taurine, Aspirin, and Sodium Lauryl Sulfate (SLS) on the Absorption of Sulfanilic Acid from Rat Stomach*

Adjuvants	% absorbed in 1 h	
None	1.4 ± 0.4 (4)	$\left. \begin{array}{c} * \\ * \\ * \end{array} \right\} *$
1 mM taurine	1.4 ± 0.1 (4)	
10 mM aspirin	4.2 ± 0.7 (4)	
10 mM aspirin + 1 mM taurine	1.4 ± 0.2 (4)	
20 mM SLS	8.3 ± 0.9 (3)	

Concentration of sulfanilic acid, 1 mM; pH 1.1. Results are expressed as the mean ± SEM followed by the number of animals in parentheses. * $p < 0.001$.

Taurine added to the mucosal solution containing 1 mM aspirin induced a marked drop in the I_{sc} (Fig. 4(c)), while no significant change was observed in the PD. The R was elevated slightly. These results indicate that the effect of taurine on the gastric mucosa in the presence of 1 mM aspirin is closely related to the ion transport function. This agrees well with the fact that the taurine-induced increase of aspirin absorption is influenced by the ionic composition of the drug solution in the lumen.¹¹⁾

Fig. 4(b) and (d) show that taurine significantly recovered the notable fall of PD and I_{sc} induced by the high concentration of aspirin. The R was also slightly increased (Fig. 4(f)). These are consistent with the fact that aspirin absorption at the high concentration was decreased by taurine.¹⁾ Table I shows the gastric absorption of sulfanilic acid, as a polar poorly absorbable model drug, in the presence of taurine, aspirin, and SLS. Ten mM aspirin increased the absorption of sulfanilic acid significantly, but the increase was cancelled by the addition of taurine. This effect of taurine is similar to the one on the absorption of 10 mM aspirin.¹⁾ It is also noticeable that SLS, which caused the large reduction of R, increased the absorption of sulfanilic acid to the higher extent than aspirin. It seems to be quite all right from these results to consider that taurine has a protective function against the gastric mucosal damage induced by the high concentration of aspirin.

To summarize our interpretation of the results, we can explain that taurine increases the gastric absorption of aspirin in relation to the ion transport function of the gastric mucosa and that taurine acts as a protective substance against the gastric damage induced by the high

concentration of aspirin.

REFERENCES

- 1) T.Kimura, K.S.Kim, and H.Sezaki: Effect of taurine on drug absorption from the rat gastrointestinal tract, *J. Pharm. Dyn.*, **4**, 35–41 (1981).
- 2) H.W.Davenport: Gastric mucosal injury by fatty and acetylsalicylic acids, *Gastroenterology*, **46**, 245–253 (1964).
- 3) H.H.Ussing and K.Zerah: Active transport of sodium as the source of electric current in the short-circuited isolated frog skin, *Acta Physiol. Scand.*, **23**, 110–127 (1951).
- 4) D.Fromm, J.H.Schwartz, and R.Quijano: Effect of salicylate and bile salt on ion transport by isolated gastric mucosa of the rabbit, *Am. J. Physiol.*, **230**, 319–326 (1976); R.Fuhro and D.Fromm: Effects of compounds chemically related to salicylate on isolated antral mucosa of rabbits, *Gastroenterology*, **75**, 661–667 (1978).
- 5) J.G.Forte, H.Helbock, and P.Saltman: A versatile apparatus for membrane transport studies, *Anal. Biochem.*, **20**, 545–547 (1967).
- 6) J.G.Forte: The effect of inhibitors of HCl secretion on the unidirectional fluxes of chloride across bullfrog gastric mucosa, *Biochim. Biophys. Acta*, **150**, 136–145 (1968).
- 7) L.S.Schanker, P.A.Shore, B.B.Brodie, and C.A.M.Hogben: Absorption of drugs from the stomach. I. The rat, *J. Pharmacol. Exp. Ther.*, **120**, 528–539 (1957).
- 8) E.Kivilaakso, D.Fromm, and W.Silen: Effects of lysolecithin on isolated gastric mucosa, *Surgery*, **84**, 616–621 (1978).
- 9) K.S.Kim, M.Kurokawa, T.Kimura, and H.Sezaki: Effect of taurine on the gastric absorption of drugs. Comparative studies with sodium lauryl sulfate, *J. Pharm. Dyn.*, **5**, 509–514 (1982).
- 10) D.Fromm: Gastric mucosal barrier, "Physiology of the Gastrointestinal Tract," ed. by L.R.Johnson, Raven Press, New York, 1981, pp.733–748.
- 11) K.S.Kim, M.Tsuji, T.Kimura, and H.Sezaki: Effect of taurine on the gastrointestinal absorption of drugs. Ionic requirement for the action, *J. Pharm. Dyn.*, **5**, 172–178 (1982).