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# OSCILLATIONS OF $H^+$ SECRETION AND ELECTROGENIC PROPERTIES IN THE ISOLATED FROG GASTRIC MUCOSA

GUNNAR FLEMSTRÖM

*Department of Physiology and Medical Biophysics, Biomedical Center, University of Uppsala, Uppsala (Sweden)*

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## SUMMARY

Oscillations of $H^+$ secretion rate, active net charge transport measured as short-circuit current and transmucosal electric potential difference with a regular frequency of one period in 45 min appeared spontaneously in the isolated frog gastric mucosa. Similar oscillations were triggered by the addition of histamine at 45-min intervals.

The spontaneous oscillations and the continuation of histamine triggered oscillations after cessation of histamine administration indicates that there was a component of slow inherent rhythmicity in the mucosa. No oscillations were obtained when a small transmucosal gradient of $Cl^-$ was used.

With the histamine-triggered oscillations the $H^+$ secretion rate was always 180° out of phase with the short-circuit current and the potential difference. This supports the hypothesis of an electrogenic mechanism for active transport of $H^+$ in the mucosa that can function at least partially independently of that for $Cl^-$.

## INTRODUCTION

It is well established that active and electrogenic transport of $Cl^-$ (refs 1,2) and $H^+$ (ref. 3) takes place in the gastric mucosa. With the isolated short-circuited frog gastric mucosa, Hogben[2,4] showed that the net $Cl^-$ transport was equal to the value of the short-circuit current ($I_{sc}$) *plus* the $H^+$ secreted. The relationship between the mechanisms for transport of these ions is less well known. Some $H^+$ secretion continued also when the $Cl^-$ in the bathing solutions was completely replaced by $SO_4^{2-}$ (ref. 5) or isothionate[6]. Further evidence for the mutual independence of $H^+$ and $Cl^-$ transport mechanisms is provided from the differential action of inhibitors. Thiocyanate and some other substrances (see Rehm[7]) inhibit $H^+$ secretion but leave the $I_{sc}$ unchanged or increased. Conversely, azetazolamide reduces $I_{sc}$ to a greater extent than $H^+$ secretion[8].

In disagreement with these results, Forte[9,10] suggested a close electric or biochemical coupling between the mechanisms for active transport of $H^+$ and $Cl^-$. Conditions leading to a decrease (anoxia) or increase (10 $\mu$M histamine) in the

Abbreviation: PD, transmucosal electric potential difference.

active component of $Cl^-$ flux produced a stoichiometric change in the rate of $H^+$ secretion. Other evidence for some metabolic coupling was provided from the finding that some short-chain fatty acids increase $H^+$ secretion without changing the $I_{sc}$ appreciably[11,12].

As a consequence of the apparently divergent data it seemed of interest to obtain further information about the relationship between the electrogenic properties and $H^+$ secretion in the gastric mucosa. In the present work this was done by measuring the electrogenic properties during a period when oscillations in the $H^+$ secretion rate were triggered by intermittent injection of histamine.

## METHODS

Frogs of the species *Rana temporaria* were kept in a tank containing tap water at a temperature of 6–10 °C. Each animal was given 125 mg of liver once or twice a week by manual force-feeding. No food was given for two days preceding an experiment.

The time for isolation and mounting of the mucosa as a membrane between two perspex chambers did not exceed 6 min. The exposed mucosal area was 1.8 $cm^2$. There was 20 ml of solution on each side which was changed 3–4 times during a 20-min period before the experiments were started. A gas lift ($O_2$–$CO_2$; 95:5, v/v) gave a satisfactory circulation of the solutions. The $O_2$ content of the gas was reduced to 40% in some experiments, the residue being 5% $CO_2$ and 55% $N_2$. This reduction of $pO_2$ has been shown earlier[13] to result in slight mucosal hypoxia. The compositions of the solutions are given in Table I. There was no transmucosal gradient for ions known to have significant permeability in the mucosa. All experiments were performed at 20±0.1 °C.

The rate of $H^+$ secretion was measured continuously[8], the pH on the secretory side being kept constant at 4.70 by infusion of a recorded volume of 15 mM NaOH

TABLE I

COMPOSITIONS (mM) OF THE NUTRIENT (pH 7.12) AND SECRETORY SIDE SOLUTIONS AND THE SOLUTION FOR TITRATION

| *Compound* | *Nutrient side* | *Secretory side* | *Titration* |
|---|---|---|---|
| NaCl | 81.6 | 80.8 | 84.4 |
| $Na_2SO_4$ | — | 10.8 | 1.5 |
| KCl | 3.2 | 4.0 | 4.0 |
| $CaCl_2$ | 1.8 | 1.8 | — |
| $MgSO_4$ | 0.8 | 0.8 | — |
| $KH_2PO_4$ | 0.8 | — | — |
| $NaHCO_3$ | 17.8 | — | — |
| Sodium butyrate | 3.0 | — | — |
| Glucose | 2.0 | — | — |
| Mannitol | — | 12.8 | 7.9 |
| NaOH | — | — | 15.0 |

solution (see Table I) from an autoburette under automatic control from a glass–calomel electrode. Short-circuit current ($I_{sc}$) was manually applied to the mucosa from an external source and recorded every 5 min from the voltage drop over a high precision resistor. The transmucosal electric potential difference (PD) was either determined every 10 min as the equilibrium open circuit potential 1–2 min after disconnection of the $I_{sc}$ or recorded continuously (open-circuit conditions). It was measured with a potentiometer recorder (Mosely 711 BM, Hewlett-Packard, Cal., U.S.A.) *via* matched calomel electrodes. The resistance of the mucosa was estimated after every recording of the PD from the equilibrium change of the open circuit potential when a fixed current (30 $\mu A/cm^2$) was passed through the mucosa in the same direction as the $I_{sc}$. The resistance values were corrected for the resistance of the solutions between the calomel electrodes.

Histamine (histamine·2 HCl dissolved in nutrient side solution) was added to the nutrient side solution (pH 7.12) either continuously or, for reasons discussed below, at 45-min intervals. In both cases histamine was first added to a concentration of 0.6 $\mu$M at time zero. When given continuously, infusion at a constant rate which gave the same amount of histamine in 1 h as the first single dose was then started immediately. This rate of infusion of histamine gave good stability of $H^+$ secretion and electric properties[13]. When given at intervals, histamine was then added every 45 min to a concentration of 0.45 $\mu$M. For infusion an apparatus with good stability properties[14] was used.

## RESULTS

When histamine was infused continuously at a constant rate it was observed that the $H^+$ secretion, $I_{sc}$ and PD varied rhythmically in some of the experiments. The form of these spontaneous oscillations was more or less sinusoidal with a regular frequency of one period in 45 min as shown in Fig. 1. In most cases the oscillations were "damped" but oscillations with continuously increasing amplitudes were also found in a few experiments. Only minor and irregular variations in the resistance appeared. Oscillations were seen when the PD was continuously reduced to zero

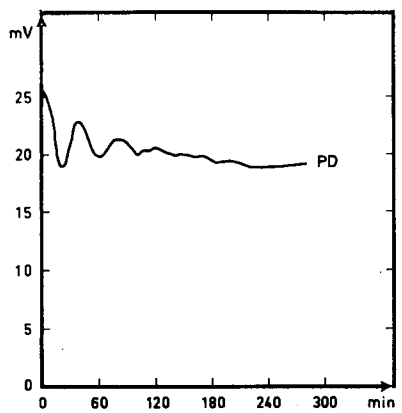


Fig. 1. The results of an open circuit experiment with continuous recording of the electric potential difference (PD). The $H^+$ secretion and the other electric properties were not measured. Histamine was infused continuously at a constant rate.

by short-circuit current as well as under open-circuit conditions. The phase relationship between the $H^+$ secretion rate and the electrogenic properties differed in the individual mucosae but the secretion rate often showed a maximum when the $I_{sc}$ and the PD were at a minimum. Oscillations of only one or two of the measured parameters were also observed.

As the frequency of the spontaneously appearing oscillations was regularly found to be one period in 45 min, histamine was given at 45-min intervals to the short-circuited mucosa in an attempt to trigger oscillations. Under these conditions, oscillations of $H^+$ secretion rate, $I_{sc}$ and PD were induced after some latency in 10 out of 14 mucosae. The resistance of the mucosa was greater in experiments without oscillations than in those where oscillations were obtained. A typical experiment is illustrated in Fig. 2. The $I_{sc}$ and the PD always showed a maximum when the secretion rate was at a minimum. The amplitude of the variations in the $I_{sc}$ was smaller than that of the variations in the $H^+$ secretion rate (both in $\mu$equiv/h per $cm^2$). A delay of approximately 10 min after one addition of histamine was usual before the increase in $I_{sc}$ and PD appeared. Oscillations with the same appearance could be triggered also when the mannitol in the secretory side solution was replaced by raffinose. When mucosal hypoxia was introduced by reduction of $pO_2$ in the solutions, both spontaneous and histamine triggered oscillations stopped.

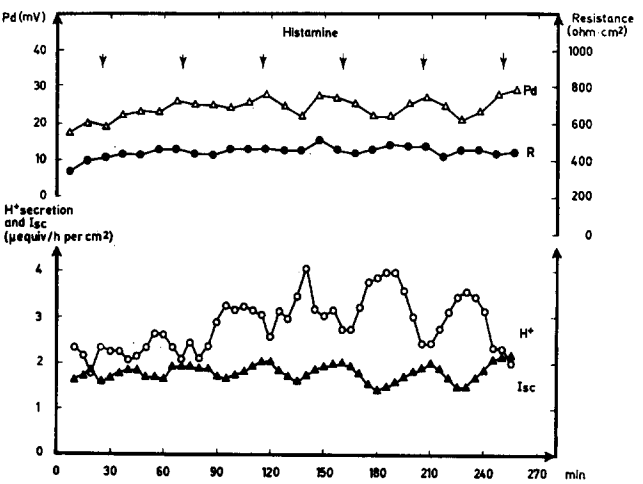


Fig. 2. The results of an experiment in which oscillations of $H^+$ secretion rate, $I_{sc}$ and PD were induced by the addition of histamine at 45-min intervals. Addition of histamine is indicated by arrows. Only irregular variations of the resistance ($R$) are seen.

The current–voltage relationship in histamine induced and spontaneous oscillations did not differ significantly from the rectilinear relationship earlier[15] found under steady-state conditions.

In some experiments all histamine treatment was terminated when oscillations had already been evoked by the administration of histamine at intervals. As can be seen in Fig. 3, oscillations in $H^+$ secretion rate and $I_{sc}$ persisted but the phase relationship changed such that the oscillations of the secretion rate were no longer 180° out of phase with those of the $I_{sc}$.

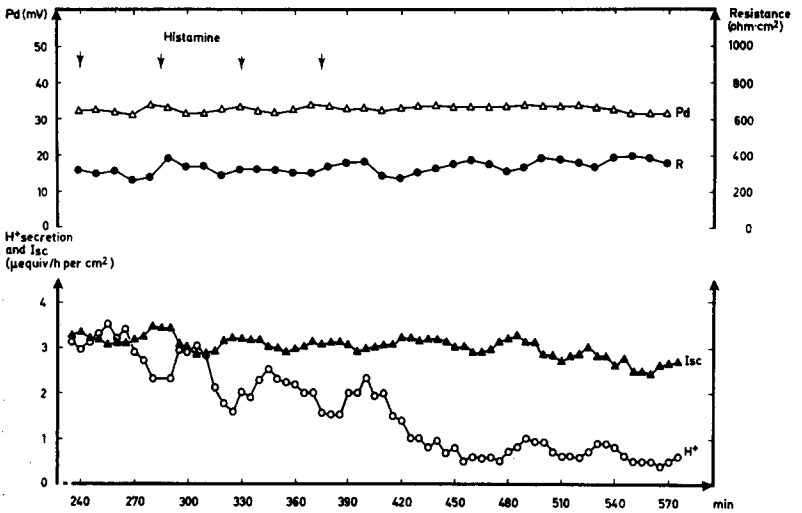


Fig. 3. The results of an experiment starting in the same way as that in Fig. 2. In this experiment the addition of histamine was discontinued.

In 9 experiments another secretory side solution was used. This differed only in that 10.8 mM of $SO_4^{2-}$ were replaced by $Cl^-$ and mannitol was excluded. There was thus a total $Cl^-$ concentration of 110 mM in the secretory side solution, while that in the nutrient side solution was still 88.4 mM. The nutrient side solution was the same as in the previous experiments. The duration of the experiments was 5 h and the same amount of histamine as in the previous experiment was given at 45-min intervals. With this $Cl^-$ concentration gradient no oscillations were observed.

## DISCUSSION

The time delay of 10 min between the administration of histamine and the start of an increase in $H^+$ secretion is about the same time as found by Kasbekar[16] for diffusion of histamine into the isolated frog gastric mucosa. The oscillations on intermittent histamine injection may thus partially reflect a dose–response relationship. The spontaneous oscillations and the continuation of oscillations despite termination of histamine administration makes it probable, however, that in addition there is an inherent component of slow rhythmicity involved in the oscillations. A time lag in the recording of $H^+$ secretion due to time necessary for passage of $H^+$ from the "site of production" to the secretory side solution can hardly have given a phase shift in view of the slow frequency of the oscillations. Oscillations of PD[17,18] and impedance[18] have been reported previously in the isolated frog skin; PD was maximal when the impedance was minimal[18].

In view of the complex composition of the gastric mucosa it is presently not possible to explain satisfactorily an inherent rhythmicity. The disappearance of oscillations on introduction of slight mucosal hypoxia may, however, indicate their dependence on tissue metabolism and they may also possibly reflect cyclic

metabolic processes in the secretory cells. Such processes are well known in several types of isolated cells[19,20]. It may also be relevant that oscillatory processes can be produced in artificial charged membranes[21,22]. It should be noted that the introduction of a comparatively small transmucosal gradient of $Cl^-$ prevented the appearance of oscillations; the ion distribution profile has been postulated as of importance for the stability of a biological membrane[23].

With the histamine-triggered oscillations there was always an inverse relationship between the $H^+$ secretion rate and both the PD and the $I_{sc}$. Such a relationship would be expected if there were an electrogenic transport of $H^+$ that changed rhythmically in magnitude without similar changes in the other electrogenic components. The results thus appear to be in disagreement with the evidence[9,10] that there is a close electric or biochemical coupling between $H^+$ and $Cl^-$ transport on histamine stimulation. The concentration of histamine used in the present experiments was, however, smaller and it has earlier (*cf.* Rehm[24]) been shown that high concentrations of histamine may stimulate both $H^+$ and $Cl^-$ transport.

Hogben[2,4] showed that $I_{sc}$ in the isolated frog gastric mucosa could account for the net transport of $Cl^-$ *minus* that of $H^+$. Evidence has since been produced that also $Na^+$ can be electrogenically transported in the frog mucosa[13,25] but this transport occurred only an $O_2$ tension lower than that used here when oscillations were obtained. Electrogenic transport of ions other than $H^+$ and $Cl^-$ in the present experiments seems therefore less likely.

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REFERENCES

1 Rehm, W. S. (1950) *Gastroenterology* 14, 401–417
2 Hogben, C. A. M. (1951) *Proc. Natl. Acad. Sci. U.S.* 37, 393–395
3 Rehm, W. S. and LeFevre, M. E. (1965) *Am. J. Physiol.* 208, 922–930
4 Hogben, C. A. M. (1955) *Am. J. Physiol.* 180, 641–649
5 Heinz, E. and Durbin, R. P. (1959) *Biochim. Biophys. Acta* 31, 246–247
6 Forte, J. G., Adams, P. H. and Davies, R. E. (1963) *Nature* 197, 874–876
7 Rehm, W. S. (1972) *Arch. Intern. Med.* 129, 270–278
8 Durbin, R. P. and Heinz, E. (1958) *J. Gen. Physiol.* 41, 1035–1047
9 Forte, J. G. (1969) *Am. J. Physiol.* 216, 167–174
10 Forte, J. G. (1971) in *Membranes and Ion transport* (Bittar, E. E., ed.), Vol. 3, pp. 111–164, Wiley, London
11 Alonso, D., Nigon, K., Dorr, I. and Harris, J. B. (1967) *Am. J. Physiol.* 212, 992–1000
12 Flemström, G. (1971) *Acta Physiol. Scand.* 82, 1–16
13 Flemström, G. (1971) *Biochim. Biophys. Acta* 225, 35–45
14 Öbrink, K. J. (1948) *Acta Physiol. Scand.* 15, Suppl. 51
15 Crane, E. E., Davies, R. E. and Longmuir, N. M. (1948) *Biochem. J.* 43, 321–336
16 Kasbekar, D. K. (1967) *Proc. Soc. Exp. Biol. Med.* 125, 267–271
17 Hashida, K. (1922) *J. Biochem. Tokyo* 1, 21–67
18 Teorell, T. (1954) *Acta Physiol. Scand.* 31, 268–284
19 Chance, B., Estabrook, R. W. and Ghosh, A. (1964) *Proc. Natl. Acad. Sci. U.S.* 51, 1244–1251

20 Hess, B. and Boiteux, A. (1968) in *Regulatory Functions of Biological Membranes* (Järnefelt, J., ed.), pp. 148–162, Elsevier, Amsterdam
21 Teorell, T. (1955) *Exp. Cell Res. Suppl.* 3, 339–345
22 Teorell, T. (1969) in *Laboratory Techniques in Membrane Biophysics* (Passow, H. and Stämpfli, R., eds), pp. 130–140, Springer-Verlag, Berlin, Heidelberg and New York
23 Teorell, T. (1971) in *Handbook of Sensory Physiology* (Loewenstein, W. R., ed.), Vol. I., pp. 291–339, Springer-Verlag, Berlin, Heidelberg and New York
24 Rehm, W. S. (1962) *Am. J. Physiol.* 203, 63–72
25 Flemström, G. and Öbrink, K. J. (1972) in *Gastric Secretion* (Sachs, G., Heinz, E. and Ullrich, K. J., eds), pp. 189–200, Academic Press, New York and London