BBA 4271

ACTION OF HISTAMINE ON THE PERMEABILITY OF THE FROG GASTRIC MUCOSA TO POTASSIUM AND WATER*

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(Received April 16th, 1963)

SUMMARY

The permeability to potassium and water of the isolated gastric mucosa of the frog, *Rana pipiens*, has been studied. Measurements were realized in spontaneously secreting and histamine ($2 \cdot 10^{-5}$ M) stimulated mucosae *in vitro*. Electrical potential differences and net potassium fluxes were measured in solutions containing known potassium concentrations. Permeability coefficients for potassium of $11.8 \cdot 10^{-6}$ and $22.6 \cdot 10^{-6}$ cm/sec were calculated for the spontaneously secreting and histamine-stimulated mucosae, respectively. A diffusion permeability coefficient for water of $0.42 \cdot 10^{-4}$ ml/cm^2·sec, was obtained for the spontaneously secreting mucosa using ^{3}H-labelled water; addition of histamine did not change the water diffusion rate. Values of $2.2 \cdot 10^{-2}$ and $3.7 \cdot 10^{-2}$ cm^4/sec per mole of glucose were obtained for the permeability coefficient for water filtration in the spontaneously secreting and histamine-stimulated mucosae, respectively. From these findings, it may be concluded (a) that stimulation with histamine increases the permeability of the mucosa to potassium and to water filtration, and (b) that the main barrier for the diffusion of water and that for filtration of water are different, the one for diffusion being insensitive to histamine at the concentration used.

INTRODUCTION

It is known that the isolated frog gastric mucosa transports Cl^- actively from its serosal to its mucosal side in excess to that secreted as HCl[1-3]. A transmucosal electrical potential difference arises from this chloride movement. The current (supplied to the mucosa from an external circuit) necessary to abolish this potential difference is called the short-circuit current and is equivalent to the difference between the chloride net flux and the H^+ secretion[2,4]. Addition of histamine induces a rise in the acid secretion and simultaneously lowers the potential difference without changing the short-circuit current[4,5].

It is also known that there exists a net flux of potassium from the serosal to the mucosal side, which is evident in open-circuit experiments, when the mucosa maintains its potential difference. Almost all this flux is considered to be passive since it practi-

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* A preliminary report of the present work was presented at the 7th Annual Meeting of the Biophysical Society, New York, February 18–20, 1963.

cally disappears when the potential difference is abolished (short-circuit condition)[2,6–8]. The influence on the net potassium flux produced by changes in the potassium concentration of the solutions bathing either side of the mucosa has been studied under control conditions and after addition of histamine[6,9]; however, no effect on this flux attributable to histamine has been observed[6]. Since the permeability coefficient for an ion depends on concentrations and potential difference in addition to fluxes, the possibility exists that some change in the permeability of the mucosa, which has not been explored, could occur during histamine stimulation. If the potassium ion is considered to move passively in water solution, a simultaneous effect of histamine on the permeability of the mucosa to water could also be expected.

Consequently two different groups of experiments were designed to study the permeability of the spontaneously secreting and histamine-stimulated mucosa. In the first group simultaneous measurements of spontaneous potential difference (in potentiometric conditions) and of net potassium flux were performed at known potassium concentrations in spontaneously secreting mucosae and after histamine stimulation. In the second group of experiments, the permeability to water was also studied in spontaneously secreting and histamine-stimulated mucosae, by measuring the water diffusion and filtration rates.

METHODS

Permeability to potassium

Simultaneous measurements of potential difference and net potassium fluxes across the isolated frog gastric mucosa were made at known potassium concentrations in order to characterize its permeability to potassium.

The frogs, *Rana pipiens*, were kept in tap water at room temperature (20°–22°) at least 1 week before the realization of the experiments. The animals were pithed, their stomach removed and opened along the small curvature, and its mucosa stripped, by blunt dissection, from the muscular coat.

The mucosa was mounted between two lucite chambers with suitable arrangements for sampling and aeration. The volume of each chamber was 5 cm^3. These chambers were similar to those described by USSING AND ZERAHN[10]. The area of the preparation exposed between the chambers was 1.13 cm^2. The chamber in contact with the serosal face of the preparation was filled with a buffered physiological solution (nutrient solution) and stirred by bubbling with O_2–CO_2 (95:5). The one in contact with the mucosal face was filled with a similar but unbuffered solution (secretory solution) and stirred by bubbling with O_2. Bubbling was regulated to obtain homogeneous mixing in each chamber within 15 sec. The composition of these standard physiological solutions, containing 4 mM of potassium, is shown in Table I. In one group of experiments nutrient and secretory solutions were used with the potassium concentration increased to 9 mM (high-potassium solutions) and in another the potassium concentration was lowered to 1 mM (low-potassium solutions). In these solutions, simultaneous changes in the sodium concentration were made in order to avoid osmotic effects.

Two agar–physiological solution bridges were located close to the mucosa to measure its potential difference. These bridges were connected to two symmetrical calomel electrodes which served as input for a vibrating electrometer (Cary Model 31).

Two other agar-physiological solution bridges entered the chambers and were used to short circuit the potential difference of the mucosa. For this purpose, a circuit between these bridges was used to supply current from a battery to the membrane in order to reduce its potential difference to zero (short-circuit conditions).

TABLE I

COMPOSITION OF THE PHYSIOLOGICAL SOLUTIONS

	Nutrient solution (mM)	*Secretory solution (mM)*
NaCl	84.6	102.1
KCl	3.2	4.0
$CaCl_2$	1.8	1.8
$MgSO_4$	0.8	0.8
KH_2PO_4	0.8	—
$NaHCO_3$	17.8	—
Glucose	5.5	5.5

The experiments lasted for about 6 h during which the spontaneous potential difference of the mucosae (open-circuit conditions) was continuously recorded. The magnitude of the short-circuit current was measured every 30 min by bringing the potential difference to zero as already described, during a time interval which never lasted more than 2 min.

The experiments consisted of three successive observation periods of 2 h each. The first and third observation periods, served as control periods, were carried out with the mucosa bathed by the standard physiological solutions. Experiments in which potential difference in the third period was not comparable to that measured during the first period were rejected. During the second, or experimental period, solutions with high, standard, or low potassium concentration were used. To estimate the net potassium flux, nutrient and secretory solutions were assayed for potassium concentration every hour using flame spectrophotometry. Comparable experiments were performed with histamine diphosphate added to the solutions during the three observation periods, to obtain a concentration of $2 \cdot 10^{-5}$ M.

Permeability to water diffusion

Diffusion of ^3H-labelled water was measured between nutrient and secretory solutions, in another group of experiments.

The unidirectional diffusion flux of water from the nutrient to the secretory side was measured by placing the isolated mucosa between chambers similar to those already described. For these measurements, the volume of each chamber was 10 cm^3. After 15 min that were allowed for equilibration in standard physiological solutions, the chamber facing the serosal side was filled with nutrient solution prepared in water labelled with 0.2 μC of ^3H per ml. Samples of 0.1 ml were taken from both chambers immediately after addition of the radioactive nutrient solution and every 30 min thereafter for 3 h. Histamine was then added to a concentration of $2 \cdot 10^{-5}$ M on both sides and the experiment continued for two more hours. ^3H activity in the samples was measured in a liquid scintillation counter (Tricarb spectrometer Model 314 DC), as described by VILLEGAS AND VILLEGAS[11].

Permeability to water filtration

Filtration was induced by creating an osmotic pressure difference across the mucosa by addition of glucose to the secretory solution. Volume changes of the nutrient solution were measured using an apparatus similar to that described by DURBIN *et al.*[12]. The isolated mucosa was mounted between two chambers. One small chamber, with a volume of 1.9 cm^3 in contact with the serosal face, was filled with nutrient solution. This solution was stirred by a glass-enclosed iron wire inside the chamber, driven by a magnet stirrer on the outside. The other chamber, in contact with the mucosal face, was filled with approx. 10 ml of secretory solution and was stirred by a stream of O_2. A 0.2-ml pipette graduated in microliters was sealed in the small chamber for direct reading of the change in volume of the nutrient solution. During the time of volume reading (about 1 min), the mucosa was fixed in a standard position against a perforated disk in the big chamber by means of a pressure of 50 cm of water applied through the measuring pipette. The rest of the time the pipette was opened to the atmosphere. Volume readings were taken every 30 min.

The experiments were performed as follows: (a) the spontaneous net volume flux, with the mucosa bathed in both sides by the standard physiological solutions, was measured for 90 min at intervals of 30 min; (b) then an osmotic pressure difference was produced by addition of 214 mM of glucose to the secretory solution, and the net volume flux was also measured for another 90 min; (c) histamine was added to a concentration of $2 \cdot 10^{-5}$ M and the measurements of the net volume flow continued for another 90 min in the presence of the same osmotic pressure difference; (d) finally, the osmotic pressure difference was suppressed by placing back the standard secretory solution but with histamine added, and the spontaneous net volume flow measured for the last 90 min.

RESULTS

Permeability to potassium

Two groups of experiments were carried out. The first group with spontaneously secreting mucosae and the second with histamine-stimulated mucosae. In one series of each group the standard physiological solutions, with 4 mM of potassium, used during the control periods were also maintained during the experimental period. In a second series of each group, high-potassium solutions, with 9 mM of potassium, were used during the experimental period. In a third series low-potassium solutions, with 1 mM of potassium were used during the experimental period.

Table II presents the values obtained for potential difference and net potassium flux, during the control periods of the two groups of experiments, expressed as means ± standard error. It may be seen that the potential difference measured in the presence of histamine are lower than those measured in the spontaneously secreting mucosae, as previously reported[4,5]. No significant differences exist between the values for the net fluxes in spontaneously secreting and histamine-stimulated mucosae. It may be seen that there is no difference in the values for short-circuit current between the two groups.

Table III presents the changes in the values of potential difference and net potassium flux produced by changing the potassium concentration simultaneously in the nutrient and the secretory solutions. The results are expressed as the mean ± standard error of the differences between the values obtained during the control and the experimental periods in 24 mucosae. It may be seen that in spontaneously secreting mucosae an increase of 5 mM in the potassium concentration of the nutrient and the secretory solutions decreased the potential difference and a decrease of 3 mM increased the potential difference. An effect of the same sign but higher in magnitude was observed in the mucosae stimulated by histamine. It may also be seen that the net potassium flux was increased at the high potassium concentration and was decreased at the low potassium concentration. However, these changes in net potassium flux secondary to changes in potassium concentration in the spontaneously secreting mucosae were of the same magnitude as those obtained in the mucosae stimulated with histamine.

TABLE II

POTENTIAL DIFFERENCE, Φ_K AND SHORT-CIRCUIT CURRENT MEASURED IN PHYSIOLOGICAL SOLUTION WITH 4 mM K^+

Values are expressed as means ± standard error.

	Potential difference (mV)	*Φ_K (mole per sec·cm² × 10^{-12})*	*Show-circuit current (μA/cm²)*
Spontaneously secreting mucosal	35 ± 2	68 ± 6	128 ± 16
Histamine-stimulated mucosae	23 ± 2	67 ± 9	116 ± 10

TABLE III

CHANGES OF POTENTIAL DIFFERENCE, Φ_K AND SHORT CIRCUIT CURRENT INDUCED BY THE CHANGE IN POTASSIUM CONCENTRATION

Values expressed as the means of the differences between the measurement performed in the control and experimental periods ± standard error.

Potassium concn. (mM)	*Spontaneously secreting mucosae*			*Histamine stimulated mucosae*		
	Δ potential difference (mV)	*Δ Φ_K (mole per cm²·sec × 10^{-12})*	*Δ short-circuit current (μA)*	*Δ potential difference (mM)*	*Δ Φ_K (mole per cm²·sec × 10^{-12})*	*Δ short-circuit current (μA)*
1	+ 5 ± 2	—51 ± 2	—12 ± 5	+11 ± 2	—49 ± 3	+ 3 ± 1
4	+ 2 ± 1	— 3 ± 9	— 7 ± 4	— 1 ± 1	0 ± 10	— 1 ± 3
9	—10 ± 2	+27 ± 2	—10 ± 2	—12 ± 1	+27 ± 4	—16 ± 8

Values for short-circuit current are also given in Table III. No significant effect on the short-circuit current was produced by the potassium concentration changes, either in the presence or in the absence of histamine.

Permeability to water

Fig. 1 presents the results of a water diffusion experiment. ^3H activity of the secretory solution, after tracer amount of ^3H had been added to the nutrient solution, is presented as a function of time. No significant dilution was observed in the nutrient solution during the time of the experiment. It may be seen that after an initial equilibration period, the diffusion flux of water labelled with ^3H became constant and was not influenced by the addition of histamine. In 13 mucosae, the average unidirectional diffusion flux was $(0.42 \pm 0.04) \cdot 10^{-4}$ ml/cm²·sec in the spontaneously secreting mucosae and did not change by the addition of histamine; a diffusion flux of (0.42 ± 0.04)·10^{-4} ml/cm^2·sec was measured during histamine stimulation.

The results of a water filtration experiment are presented in Fig. 2. Volume changes of the nutrient solution are represented as a function of time. An initial net spontaneous flux of water from the nutrient to the secretory side was observed in the absence of any osmotic pressure difference. When an osmotic pressure difference was produced by adding 214 mM of glucose to the secretory solution, the net water flux towards the secretory solution was increased and a further increase of this flux was observed during histamine stimulation. A net spontaneous flux of water was also observed in the presence of histamine, when the osmotic pressure difference was suppressed by placing back the standard physiological solution. This spontaneous flux was not different from the spontaneous flux observed before addition of histamine. This may be seen in Table IV which presents the mean ± standard error of the net fluxes measured in 13 mucosae. The osmotically induced water flux was calculated by subtracting the spontaneous flux (secretory solution isotonic) from the flux observed after addition of glucose to the secretory solution to obtain a concentration

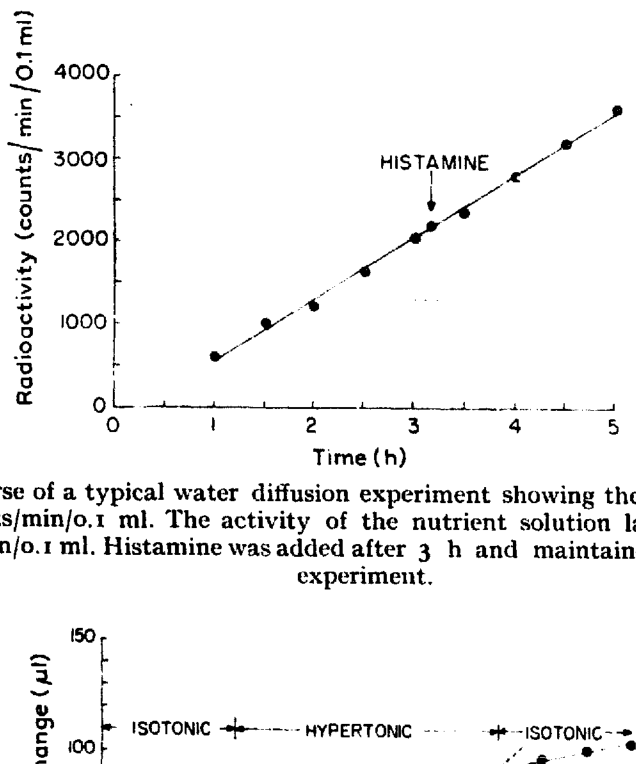

Fig. 1. Time course of a typical water diffusion experiment showing the activity of the secretory solution in counts/min/0.1 ml. The activity of the nutrient solution labelled with ^{3}HHO, was 49·10^3 counts/min/0.1 ml. Histamine was added after 3 h and maintained until the end of the experiment.

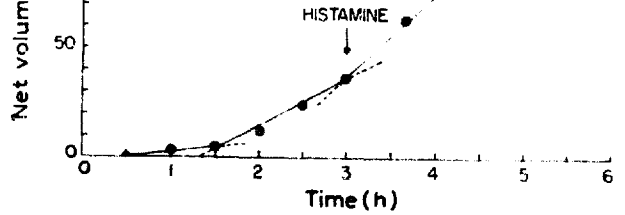

Fig. 2. Time course of a typical water filtration experiment showing the net volume change of the nutrient solution. Hypertonic secretory solution (214 mM of glucose added) was used between 1.5 and 4.5 h. Histamine was added after 3 h and maintained until the end of the experiment.

of 214 mM (secretory solution hypertonic). The osmotically induced water flux of the histamine-stimulated mucosae is 1.7 times that of the spontaneously secreting mucosae.

TABLE IV

NET WATER FLUX

Values expressed as means ± standard error.

	Secretory solution		*Osmotically induced flux* ($\mu l/cm^2 \cdot h$)
	Isotonic ($\mu l/cm^2 \cdot h$)	*Hypertonic* ($\mu l/cm^2 \cdot h$)	
Spontaneously secreting mucosae	8.0 ± 1.8	25.3 ± 2.9	17.3 ± 2.7
Histamine-stimulated mucosae	8.6 ± 2.3	37.3 ± 2.7	28.7 ± 2.5

DISCUSSION

Permeability to potassium

The rate of passive movement of an ion through a membrane may be described as follows, by USSING's Eqn. 4 (ref. 13):

$$\Phi = \frac{u'C}{zF}\left[\frac{RT\,\mathrm{d}\ln C}{\mathrm{d}x} + zF\,\frac{\mathrm{d}\Psi}{\mathrm{d}x}\right] \qquad (1)$$

in which: Φ is the unidirectional flux of the ion per unit area, u' is the mobility of the ion in the membrane, C is its concentration, and z and F have their usual meaning. The terms ($RT\,\mathrm{d}\ln C/\mathrm{d}x$) and ($zF\,\mathrm{d}\Psi/\mathrm{d}x$) represent the diffusion and electrical forces acting upon 1 mole of the ion in the x direction perpendicular to the membrane surface.

P, the permeability coefficient for an ion which moves passively through a membrane is defined[14] by the following equation:

$$P = \frac{RTu'}{zF\Delta x} \qquad (2)$$

where Δx is the membrane thickness. Thus, in order to relate the permeability of the membrane to the measured net flux and to the potential difference, Eqn. 1 can be integrated following GOLDMAN[15] and HODGKIN AND KATZ[16], with the following assumptions: (a) that the electrical field is constant through the system, (b) that the membrane acts as a homogeneous face so that the mobility of the ion is constant, and (c) that the ions move in the membrane under the influence of an electrical gradient in a manner similar to that in free solutions. The following expression is obtained for a monovalent cation:

$$\Phi_n = \frac{PFE}{RT} \cdot \frac{C_1 - C_2 e^{EF/RT}}{1 - e^{EF/RT}} \qquad (3)$$

in which Φ_n is the net ion flux, E is the potential difference measured across the membrane, C_1 and C_2 are the ion concentrations in the solutions bathing each side of the membrane.

As has already been mentioned in the introduction, in the frog gastric mucosa the potassium ion is considered to move passively under an electrochemical potential gradient[2,6-8]. Since we have measured the net potassium flux (Φ_K) and the potential difference across the mucosa (E) at known potassium concentrations (C_K), the permeability coefficient for potassium (P_K) may be calculated. Under our experimental conditions the potassium concentration in the nutrient solution was equal to that in the secretory solution at the beginning of each experimental period and the concentration change in either solution during the experimental period was smaller than 2 %. Therefore, it is justified without introducing an error bigger than 1.5 % to use the following expression, which is obtained from Eqn. 3, taking $C_1 = C_2 = C_K$:

$$\Phi_K = P_K \cdot C_K \, FE/RT \tag{4}$$

Fig. 3 represents Φ_K, the measured values for the net potassium fluxes in mole/cm²·sec, plotted against $C_K FE/RT$ in mole/cm³. Thus, the slope of the line is equal to P_K, the potassium permeability coefficient. It may be seen (a) that P_K is independent of C_K between 1 and 9 mM, the lowest and the highest concentrations used in the present work, indicating that between these limits the sites available for potassium diffusion are not saturated. The possibility of reaching saturation of these sites[17] at higher potassium concentrations could be the explanation for the steady value for the electrical resistance of the mucosa obtained by HARRIS AND EDELMAN[18] between 12 and 40 mM of potassium. (b) That P_K for the histamine-stimulated mucosae is 1.9 times that of the spontaneously secreting mucosal. P_K values of 11.8·10⁻⁶ cm/sec for the spontaneously secreting mucosae and 22.6·10⁻⁶ cm/sec for the histamine-stimulated mucosal have been determined. (c) That the values for C_K (FE/RT) which correspond to zero Φ_K in Fig. 3 are not different from zero. This is in agreement with the view that most of the net potassium flux is passive.

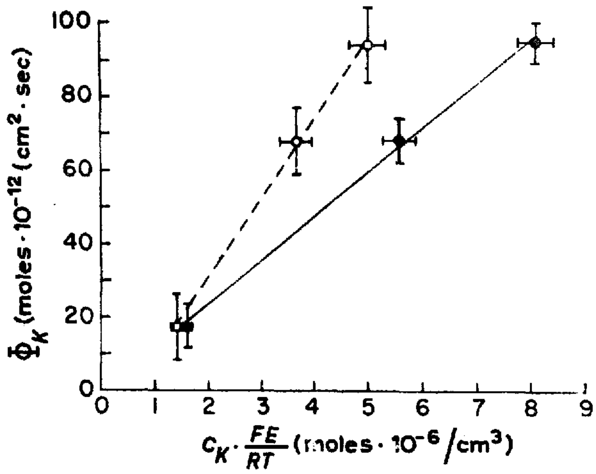

Fig. 3. The permeability coefficient for potassium is the slope of the line obtained by plotting Φ_K against C_K (EF/RT) as described in the text. The solid line represents observations in spontaneously secreting mucosae and the dotted line those in histamine-stimulated mucosae. Both lines were fitted by the least squares method.

Permeability to water

The permeability coefficient for water diffusion through the mucosa is 0.42·10⁻⁴ cm/sec in the spontaneously secreting mucosae, the units being a measure of the volume of water, in cm³, that will cross by simple diffusion 1 cm² of mucosa in 1 sec. No change of the permeability coefficient for water diffusion was produced by histamine.

The filtration permeability coefficient for water, is calculated to be $2.2 \cdot 10^{-2}$ cm^4 per sec per mole of glucose, for the spontaneously secreting mucosa, and $3.7 \cdot 10^{-2}$ cm^4 per sec per mole of glucose, for the histamine-stimulated mucosae. Therefore, the osmotic permeability of the histamine-stimulated mucosal is 1.7 times that of the spontaneously secreting mucosae.

The observation that histamine produces a significant increase in the permeability to water filtration and has no influence in the permeability to water diffusion, suggests the existence of at least two different barriers arranged in series. A similar effect on water permeability is known to be produced in the amphibian skin by antidiuretic hormone[19].

Diffusion experiments of graded-size non-electrolyte molecules allow calculation of an equivalent pore radius for the gastric mucosa between 3 and 4.5 Å not affected by histamine stimulation[23]. Larger values can be calculated by combining the water diffusion and filtration permeability coefficients. Discrepancy between the equivalent pore radius calculated from the water permeability data and that obtained from the permeation of graded-size molecules have been reported in other tissues, and explained in terms of double barrier arrangements[19,21,22]. A similar model for the gastric mucosa would also explain, as already pointed out, the discrepancy found on the effect of histamine on water filtration but not on water diffusion.

In summary it has been found (a) that the permeability for potassium of the mucosa is increased 1.9 times during histamine stimulation, (b) that the diffusion of water through the mucosa is not affected by histamine stimulation and (c) that the filtration of water is increased 1.7 times during histamine stimulation.

Therefore, the diffusion of water and of non-electrolyte molecules appears to be limited by a histamine-insensitive barrier while water filtration and potassium diffusion appears to be limited by a histamine-sensitive barrier. Since no action of histamine on the short-circuit current has been observed, the present results suggest that histamine action may be explained by an effect on the limiting barriers. However, they do not exclude a simultaneous effect of the histamine on the active mechanism responsible for the Cl^- and H^+ secretion, as previously proposed from the observation that no change was observed in the ratios Cl^-/O_2 and H^+/O_2 during histamine stimulation[23].

ACKNOWLEDGEMENTS

The author wishes to thank Dr. R. VILLEGAS and Dr. G. WHITTEMBURY for their comments on the manuscript. The valuable technical assistance of Miss L. SANANES, Miss G. NALSEN and Mr. J. MORA are appreciated.

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