Effects of increased $O_2$ and $CO_2$ on acid secretion by dogfish gastric mucosa in vitro

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KIDDER, GEORGE W. III. Effects of increased $O_2$ and $CO_2$ on acid secretion by dogfish gastric mucosa in vitro Am. J. Physiol. 231(4): 1240–1245. 1976.—The gastric mucosa of the dogfish (Squalus acanthias), as usually prepared for in vitro chambered experiments, shows a secretory rate ($J_s$) of about 2 μeq/cm²·h, but a potential difference (PD) of zero. Raising $P_{CO_2}$ from 0.06 to 0.1 atm increases $J_s$ by 40% and causes the development of a PD of about 2 mV, mucosal surface positive. Increasing $P_{O_2}$ from 0.9 to 1.9 atm in a hyperbaric chamber (at constant $P_{CO_2} = 0.1$ atm) doubles $J_s$ and increases PD to 5 mV. Transepithelial resistance falls by 20% at high $P_{O_2}$. It appears that the dogfish gastric mucosa, like that of the frog, is rate limited by $CO_2$ diffusion into the tissue from the usual 5% mixture and is also rate limited by the usual $O_2$ levels (unlike the frog), presumably due to its thicker structure and higher $O_2$ consumption. The mucosal-positive PD, which is reversed from all other mucosae studied, is readily explained by separate electrogenic $H^+$ and $Cl^-$ pumps, but less readily by schemes embodying a neutral HCl pump. It is not yet known whether the hyperbaric conditions are sufficient to ensure $O_2$ sufficiency.

carbon dioxide; diffusion; hyperbaric; potential difference, resistance; Squalus; stomach; unstirred layers

GASTRIC MUCOSAE from a number of species can be successfully freed of their external muscle coat, mounted in an Ussing-type chamber, and shown to secrete HCl at reasonable rates. With the exception of the mucosae from elasmobranchs, all of these tissues develop large (>10 mV) potential differences oriented with the mucosal surface negative to the serosal, which seems to be due to an electrogenic active transport of $Cl^-$ into the lumen. In the dogfish (Squalus acanthias) the potential difference is reported to be zero (6) or vanishingly small, with a slight tendency for the mucosal surface to be positive to the serosal surface (14). Thus, this tissue has been cited as a prime example of a coupled, or unitary, HCl pump, in which the acid secretory system, always transporting one $H^+$ and one $Cl^-$ together, produces no potential. According to this theory, those tissues that produce potentials do so by virtue of possessing not only the coupled HCl pump, but also an additional, electrogenic $Cl^-$ pump which likewise transports $Cl^-$ into the lumen.

In the gastric mucosa of the bullfrog, we have shown (24) that gassing the solutions with 5% $CO_2$ (at 1 atm total pressure) does not provide sufficient diffusion gradient to supply the cells with their required $CO_2$, and that significantly higher secretory rates are found when 10% $CO_2$ is used. Calculation (16) and experiment (25) show that for $O_2$, the usual gas mixtures (90% $O_2$) are sufficient, although there is little margin for error. Since the dogfish gastric mucosa is somewhat thicker than that of the bullfrog and has a comparable secretory rate, it was thought worthwhile to inquire whether this tissue might also be rate limited by $CO_2$ diffusion and whether it was limited by $O_2$ as well. It seemed likely that removal of these limitations on acid secretion due to external conditions might result in increased secretory rates and the development of a significant potential difference. As the results will show, these expectations were realized.

Some of these results have previously been presented in condensed form (18, 21).

METHODS

Dogfish (Squalus acanthias) were caught on baited hooks in Frenchman Bay, and stored in "live cars" attached to a dock for up to 3 days before use. Gastric mucosa from the anterior part of the stomach was dissected free of superficial muscle layers and stretched as a flat sheet (3.14 cm² area) between two fluid-filled chambers. The steps between the sacrifice of the fish and the establishment of circulation and aeration in the chamber took about 15 min, and the tissue was kept in ice-cold oxygenated Ringer during this time.

The chamber system used is diagrammed in Fig. 1. For experiments at atmospheric pressure, each reservoir was fitted with a glass tubing "cooling coil" in series with the fluid stream, which was immersed in water in an insulated bath. Manual addition of ice or warm water was used to keep the reservoir temperature at 18°C. Potential difference (PD) was recorded by connecting the PD electrodes to a high-impedance recorder and is reported referenced to the mucosal solution as zero. Resistance was measured by applying a 20-μA current pulse to the Ag/AgCl electrodes and measuring the resulting PD deflection. Resistances were corrected for the chamber resistance of 8.8 Ω (27.6 Ω·cm²) due to the fluid between the electrode tips. Acid secretory rate ($J_s$) was measured by the pH-stat method, using a Radiometer titrator and titrating to pH 4.5 with NaOH. At the end of each experiment, the titrator was calibrated by noting the deflection produced by the addition of 1 or 2 μeq HCl to the mucosal solution, and the offset of the
PD electrodes was determined by placing them both in the mucosal solution.

For experiments under hyperbaric conditions, the apparatus shown in Fig. 1 was placed inside a pressure cell. Gas-tight leads were provided for the PD, current, and pH electrode wires, and the titrant hose was run through a similar connector. In addition, a "spillover" bottle was used in series with the gas line to maintain a constant 10 cmH₂O pressure in the gas system with respect to the atmosphere in the cell, which maintained a constant bubbling rate in the tissue system. All of the gas input to the pressure cell was via this line, and the outflow from the pressure cell was adjusted to maintain constant pressure (~45 kg) metal chamber could not change temperature rapidly.

Since the contents of the titrator hose are under pressure, it was necessary to use a pressure-tight syringe (Hamilton 1001LL) on the titrator and to fit this line with a pressure-tight valve to allow refilling of the titrator syringe. This system was inspected frequently for leaks (which would raise the apparent secretory rate), but none was found. Temperature was not controlled in hyperbaric experiments; however, the massive (>45 kg) metal chamber could not change temperature rapidly.

In control experiments, the voltage between the PD electrodes immersed in the same solution was found not to change with pressure, and the output of the pH electrode was likewise not a function of pressure. There was, however, some electrical leakage between the pH electrode circuit and the current sending electrodes; in consequence, resistance measurements and measurements of Jₚ could not be made in the same experiment.

The solutions used were those of Hogben (6). The serosal solution contained, in millimoles per liter: NaCl 220, NaHCO₃ 30, KCl 10, CaCl₂ 5, MgCl₂ 2, Na₂HPO₄ 1, and glucose 25. Carbachol ([2-hydroxyethyl]trimethyl ammonium chloride carbamate, 2.5 × 10⁻⁴ M) was always added to this solution as a secretory stimulant (8) at the beginning of the experiment. The mucosal solution was similar but unbuffered, containing, in millimoles per liter: NaCl 250, KCl 10, CaCl₂ 5, and MgCl₂ 2. This solution did not contain carbachol or glucose.

These solutions were gassed with various mixtures of O₂ and CO₂, depending on the conditions required, using the same gas for both surfaces. At atmospheric pressure, 5, 10, and 20% CO₂ were used, with the balance of the mixture being O₂. In the hyperbaric experiments, 10% CO₂/90% O₂ was used at atmospheric pressure (1 atm), whereas 5% CO₂/95% O₂ was used at 15 psig (2 atm), which maintains the CO₂ at 0.1 atm while raising the partial pressure of O₂ to 1.9 atm. In some experiments, N₂ replaced the O₂ to investigate the effects of frank anoxia.

Two groups of experiments were conducted during August and September of 1974 and 1975. In the 1st yr, investigations were made of the effect of CO₂ at essentially constant O₂ (0.9-0.95 atm) which did not require the hyperbaric cell. For these experiments, the experimental periods lasted 1 hr, and the secretory rate reported was determined during the last 30 min of the period, with the reported PD taken as that of the midpoint of this period (45 min). During the 2nd yr, the hyperbaric system was used to investigate the effects of changes in oxygen tension. Since the tissue seems to respond slowly to changes in oxygenation, 2-h periods were used, with the last 30 min taken as representing the steady state, as before. Otherwise, every attempt was made to keep the conditions in the hyperbaric system as similar as possible to the experiments from the previous year.

Since it was suspected that the usual amount of O₂ (0.9 atm) might be insufficient, and perhaps cause tissue damage, all hyperbaric experiments were started with 15 psig (1.9 atm O₂), and the lower pressure was run subsequently. In most cases a third 2-h period was allowed, again at 2 atm. In the experiments of the first summer, 5 and 10% CO₂ were presented in random order.

**RESULTS**

Figure 2 shows a single experiment from the 1974 series in which 5% CO₂/95% O₂ is compared to 10% CO₂/90% O₂. Upon changing from 5 to 10% CO₂, Jₚ increased and PD becomes more negative (mucosa positive). These changes are reversed by return to 5% CO₂, and are repeatable.

For purposes of tabulation, the average acid secretory rate during the last 30 min of each period was taken as the steady-state value for that condition, whereas the PD and resistance at 45 min (the midpoint of the acid secretory period) were taken as representative values. There is some indication that steady state may not have been reached in 1 h, and thus the changes to be reported are probably underestimates of the true effect.
The differences are thought to be slight, however.

In a series of seven tissues, alternated in this manner between 5 and 10% CO₂, a total of 15 periods in each condition were observed. The acid rate, \( J_H \), was 1.89 ± 0.16 μeq/cm²·h (mean ± SE) in 5% CO₂ and rose to 2.71 ± 0.19 in 10% CO₂, a 60% stimulation which is significant at the 1% level by the Student t test. Likewise, the PD changed from −0.6 ± 0.5 mV in 5% CO₂ (not different from zero) to −1.8 ± 0.6 mV in 10% CO₂, which is significantly different from zero \((P < 0.01)\). Tissue resistance was 425 ± 46 Ω·cm² in 5% CO₂ and 426 ± 38 Ω·cm² in 10% CO₂; the difference is clearly negligible.

Thus, elevated CO₂ stimulates gastric acid secretion in this tissue and causes the development of a mucosal-positive PD which is small but significant. This PD responds to changes that affect the secretory rate, as shown in Fig. 3. Both anoxia and SCN⁻, which reduce acid secretion, also shift the PD toward zero. Thus the PD which is developed in the presence of sufficient CO₂ seems to be related to the acid secretory activities. In a few experiments, the use of 20% CO₂/80% O₂ was found to inhibit \( J_H \) as compared to the 10% CO₂ condition. In view of the O₂ data below, it is not clear whether this inhibition is due to excess CO₂ or insufficient O₂.

In another series of experiments performed during August and September of 1975, the effects of increased \( P_O_2 \) were examined at a constant \( P_CO_2 \), using the hyperbaric chamber. A typical experiment is shown in Fig. 4. The tissue was mounted, gassing started (10% CO₂), carbachol added, and the tissue chamber placed in the hyperbaric system. At time 0, the hyperbaric chamber was sealed, the gas changed to 5% CO₂, and the pressure raised to 15 psig, giving a \( P_CO_2 \) of 0.1 atm as before, but with \( P_O_2 \) of 1.9 atm. Secretory rate rises slowly, and after considerable delay, to a value much higher than that observed at atmospheric pressure, whereas PD becomes more negative. Changing to 0.9 atm O₂ reverses these changes, and further periods of 1.9 atm O₂ (not shown) will again produce stimulation.

Acid secretory rate averages for the last 30 min of the 2-h periods, with the corresponding PD values, were obtained. These results are shown in Fig. 5, along with the data from 1974. Note the large increase in both \( J_H \) and PD which occurs in 1.9 atm O₂ and the smaller increase upon changing from 0.05 atm CO₂ to 0.1 atm at constant O₂.

In a separate series of experiments, PD and resistance were measured as the \( P_O_2 \) was changed from 1.9 to 0.9 atm. For these experiments, \( J_H \) was not measured, and the buffered serosal solution was used on both sides to...
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maintain mucosal pH and provide identical concentrations in both solutions. The results are shown in Fig. 6. Elevated PO\textsubscript{2} causes a statistically significant rise in PD and a fall in resistance, again with a slow time course.

The change observed in the average J\textsubscript{H} and PD implies that these two measures might be correlated. Figure 7 shows the relationship between J\textsubscript{H} and PD for all tissues in the hyperbaric series. The regression line corresponds to the equation J\textsubscript{H} = 3.38 - 0.66(PD), and the correlation coefficient, slope, and intercept are all significantly different from zero. We are thus justified in maintaining that J\textsubscript{H} and PD are correlated in individual tissues and probably reflect changes in the same underlying processes.

**DISCUSSION**

It seems clear from these data that the acid secretory rate of the dogfish gastric mucosa in vitro, like that of the bullfrog under similar conditions, is rate limited by CO\textsubscript{2} availability at a partial pressure of 0.05 atm and that the rate is significantly higher at 0.1 atm CO\textsubscript{2}. In the latter condition, the tissue shows a small potential difference, oriented mucosal positive to serosal, which is absent at the lower CO\textsubscript{2}. This result is consistent with the hypothesis advanced to explain the similar finding in bullfrog gastric mucosa (24). Since these tissues consume CO\textsubscript{2} to produce the bicarbonate excreted from the serosal surface (29), when the rate of acid secretion exceeds the rate of metabolic CO\textsubscript{2} production, a net flow of CO\textsubscript{2} into the cells is required. This flow is driven across the serosal connective tissue and across the barrier formed by mucus and unstirred fluid on the mucosal face, by the CO\textsubscript{2} concentration gradient, which is largely determined by the partial pressure of CO\textsubscript{2} in the bathing solutions. For both frog and dogfish, it would appear that 0.05 atm CO\textsubscript{2} in the bathing solutions does not provide a sufficient gradient, and the secretory rate is thus limited by CO\textsubscript{2} availability at this pressure.

In the bullfrog gastric mucosa, calculations have shown that, in the absence of large (~1 mm) unstirred layers in the bulk solution, oxygen diffusion through the tissue will be sufficient to support the tissue respiration (16). Experiments in which the PO\textsubscript{2} was varied below the normal value show that, for bullfrog, the secretory rate starts to decline sharply below about 0.8 atm O\textsubscript{2}, but that increases up to 1.9 atm (hyperbaric experiments) do not cause increases in J\textsubscript{H} or PD (25). Thus, the calculations are in agreement with experimental data for the bullfrog system.

The gastric mucosa of the dogfish is somewhat thicker than that of the bullfrog (0.1 vs. 0.08 cm in our hands). The respiratory rate is unknown; if it were the same as that of the bullfrog, and the distribution of respiring volume were similar, one would expect that this tissue would be in a satisfactory state of oxygenation at normal pressure. The experiments do not bear out this conclusion; a considerable increase in J\textsubscript{H} and PD, with a drop in resistance, are associated with an increase in PO\textsubscript{2} at constant PCO\textsubscript{2}. It would appear that the respiratory rate of the dogfish gastric mucosa must be higher than that of the bullfrog.

In the dogfish gastric mucosa, when it was believed to secrete acid without generating a PD (6 15), the most
logical explanation was that, in this tissue, a neutral pump existed without the additional electrogenic Cl pump. If this HCl pump exists in one tissue, evolutionary considerations would suggest its existence in higher forms as well. (Rehm (28), however, reports small PD’s in other elasmobranchs.) We now find that with adequate oxygen and CO$_2$ supplies that there is in fact a PD across the dogfish gastric mucosa, and thus if a neutral pump exists, there must also exist another electrogenic pump responsible for this PD. If only H$^+$ and Cl$^-$ are involved in active transepithelial transport in this tissue, the most simple form of the HCl pump hypothesis would require that either an electrogenic H$^+$ pump exists in parallel with the HCl pump or that an electrogenic Cl$^-$ pump is oriented to transport Cl$^-$ from mucosal to serosal surface. Both of these suggestions seem unlikely and raise more problems than they solve.

However, if the electrogenic hypothesis is true, no such problems arise. The observation of a negative PD in the dogfish gastric mucosa would be explained by postulating a H$^+$ pump which was somewhat more potent than the parallel Cl$^-$ pump, instead of the converse as postulated for other organisms. Thus, only a quantitative change in relative pump activities is required to account for the observed PD. Sodium would seem to be a logical candidate, especially since this ion is actively transported in the mammalian gastric mucosa (16, 27), and, more interestingly, in the hypoxic frog gastric mucosa (2, 3). However, in all of these cases, Na$^+$ transport is from lumen to blood, and thus would not account for a mucosal-positive PD. It remains possible that Na$^+$ or some other ion is responsible for the PD in the dogfish, and the present experiments do not address this point.

Regardless of the origin of the observed PD, the observation of marked increases in J$_H$ with elevation of both CO$_2$ and O$_2$ indicates that this tissue, as previously studied, was rate limited by external conditions. Thus, previous data from this tissue must be viewed with caution, particularly with respect to the ratio of H$^+$ to Cl$^-$ transport and the implications for the neutral pump. Whereas it may well be that the hyperbaric conditions have not resulted in a tissue which is not rate limited by external conditions, it is clear that the provision of less O$_2$ or CO$_2$ will be insufficient.

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