CARBON MONOXIDE INSENSITIVITY OF THE GASTRIC MUCOSA OF RAJA ERINACEA

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We have previously shown that the chambered gastric mucosa of the skate secretes acid at high rates into its mucosal-facing solution, that this secretion is dependent upon oxygenation, and that the terminal oxidase inhibitor NaN_3 will abolish acid secretion. (Kidder and Kidder, Bull. MDIBL 25:40, 1985) Spectrophotometric experiments showed that even in the presence of high concentrations of N_3^-, most of the cytochromes could still respond to O_2-N_2 pulses. Since cytochrome oxidase (cytochrome $a+a_3$) is completely reduced by N_3^-, this suggests that an alternate route of reaction of oxygen with the tissue is available.

In the gastric mucosa of the bullfrog, experiments with carbon monoxide, another terminal oxidase inhibitor, show that this inhibitor does not produce complete inhibition of acid secretion at a CO/O_2 of 6 in the solutions, which should give a CO/O_2 of 20 or more at the oxidase (Kidder, Am. J. Physiol. 238:G197, 1980). These experiments require hyperbaric conditions, since the partial pressure of O_2 in the solutions must remain above 0.8 atm to prevent inhibition by hypoxia alone. We have modified the hyperbaric spectrophotometer to permit its use at pressures up to 7 atm, and have repeated these experiments on the skate gastric mucosa, with the addition of spectrophotometric measurements of cytochrome redox states.

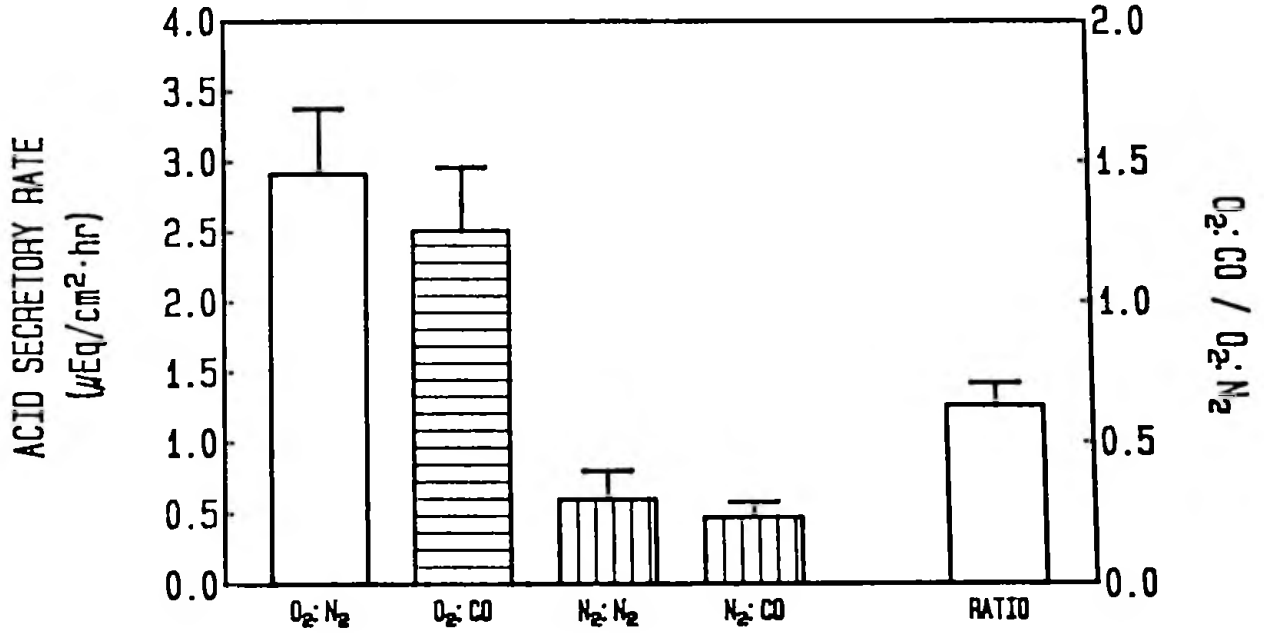

Figure 1. Response of acid secretory rate to CO. Mean ± SE. O_2:N_2 (control, N=27 tissues), O_2:CO (CO replaces N_2, N=19), N_2:N_2 (N_2 replaces O_2, N=13) and N_2:CO (N_2 replaces O_2 and CO replaces N_2, N=14). CO/O_2 is the average of the ratio in O_2:CO to that in O_2:N_2, N=19. Both anoxic conditions are significantly different from control ($P<0.01$); O_2:CO is not different ($P>0.05$). The ratio is different from 1 ($P<0.01$).

A standard elasmobranch Ringers was used on the serosal surface, and a bicarbonate-free modification of this solution on the mucosal surface. Gas mixtures were constructed by pressure in "propane" tanks to a total pressure of 300 psig from standard commercial cylinders. The control gas consists of 13.5% O_2, 1.5% CO_2, 1.7% CO, and 83.3% N_2. When used at a total pressure of 7 atm (90 psig), this gives partial pressures of 0.945 atm O_2 and 0.105 atm CO_2, consistant with the pressures employed in non-hyperbaric experiments. The small concentration of CO is used to inhibit spectrophotometric response from the residual hemaglobin. This gas is termed "O_2:N_2" or "control". O_2 was replaced by N_2 for anoxic conditions (N_2:N_2), and N_2 by CO to obtain the high-CO condition (O_2:CO). Both replacements together give anoxia with high CO (N_2:CO). The tissue was mounted in a standard Ussing chamber (4.91 cm^2 exposed area) as previously described, inserted into the hyperbaric system and sealed. Control of input and output gas flow rates allows maintaining preassure at the desired level. Electrical signals for measurement of pH and PD exit the hyperbaric chamber through gas-tight fittings.

Acid secretion was measured by pH-stat of the mucosal solution at a pH of 5.0, maintaining this pH by addition of NaOH. Under control conditions (O_2:N_2) the acid secretory rate was 2.91 $\pm$0.47 uEq/cm^2·hr in 27 tissues, consistant with previous measurements. When N_2 was replaced by CO (raising the partial pressure of CO to 5.95 atm (CO/O_2=6.30), the secretory rate declined to 2.51 $\pm$0.47 uEq/cm^2·hr in 19 tissues; this difference is not statistically significant by t-test. When the rate in O_2:CO was normalized by division by the control rate for that tissue, to remove intra-tissue variations, the average ratio was 0.63 $\pm$0.08, which is significantly different from both 1 and 0. Removal of O_2 in the presence or absence of CO drastically lowers the secretory rate, as expected.

These observations are graphed as Figure 1. It is clear that in the skate as in the frog, CO at this partial pressure only partially inhibits gastric acid secretion.

For these same tissues, reduced minus oxidized difference spectra were obtained in various conditions, using the dual wavelength spectrophotometer described by Kidder and Blankemeyer (J. Biol. Physics 5:119, 1978), as modified to permit scanning spectra and to allow spectra to be obtained from the tissue under hyperbaric conditions. The latter modification uses optical "light pipes" to lead the light from the monochromators to the tissue, and from the tissue to the photomultiplier. Representative spectra are shown as Figure 2.

A summary of the results is graphed as Figure 3. Replacing N_2 by CO has definite effects on the spectra; the largest percentage responses are those of cytochrome b and flavoprotein, which are partially reduced by CO. Cytochrome oxidase (cytochrome aa_3) appears to become oxidized, although some unexpected spectral shifts may indicate the presence of an unknown component responsible for this apparant oxidation. Cytochrome c does not change significantly ($P>0.05$).

When a nitrogen pulse is administered in the presence of high CO, all 4 components become reduced. In each case, the amount of change is less than that in the absence of CO, and these differences are significant ($P<0.05$). Unlike the case of azide inhibition, the cytochrome oxidase component still

responds to N_2 - O_2 pulses in the presence of CO, showing that it (or some component with a similar spectrum) is still free to respond to oxygen.

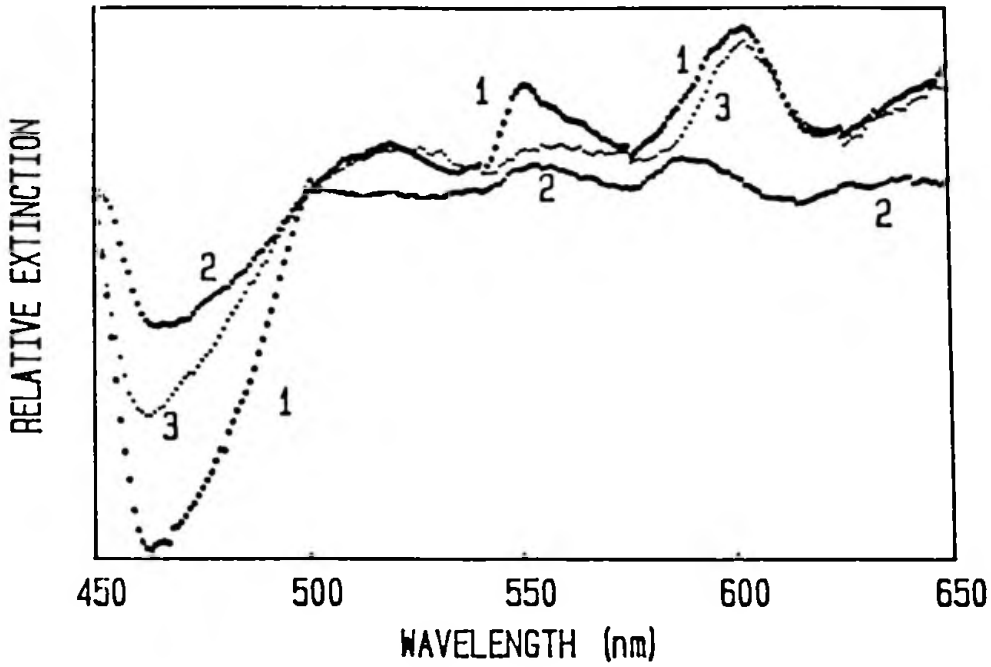

Figure 2. Representative cytochrome spectra. "1" is N_2 - O_2 difference spectrum in low CO, showing peaks at (nm) 465 (flavoprotein), 550 (cyto. c), 564 (cyto. b) and 605 (cyto. $a+a_3$). The difference spectrum between oxygenated tissues in the presence (O_2:CO) and absence (O_2:N_2) of high CO is shown as "2"; note that most of the cytochromes are little changed. "3" is the N_2 - O_2 difference spectrum in the presence of 5.95 atm CO; note that while b and c are quite reduced in O_2:CO, flavoprotein is more oxidized and $a+a_3$ nearly fully oxidized in this tissue. The summary data were derived from spectra of this type.

Two potential problems should be kept in mind in interpreting these data. First, it is possible that the cytochrome oxidase is not inhibited by a CO/O_2 of 6.3 in the bathing solutions. It was not possible to increase this ratio without either lowering the O_2 partial pressure (which would inhibit secretion itself) or raising the total system pressure, which could not be done with the available gas control equipment. Therefore, the observed secretion in the presence of CO might not indicate an alternate oxidase, but could indicate a conventional oxidase with a much lower binding coefficient for CO than the standard. Second, the spectral measurements assume the existance of isosbestic points in the spectrum. The values for these points of no cytochrome change are taken from conventional cytochrome systems, and may not be accurate if an unknown pigment is present. This could alter the quantative results obtained.

In the absence of oxygen, the entire cytochrome system should be reduced regardless of the other gasses in the system. If we assume that in the presence of oxygen the cytochromes are in an oxidized steady state, the N_2 - O_2 spectra obtained in the absence of CO should reflect the total active

cytochromes in the tissue. The addition of CO would then cause a partial reduction of the cytochromes; subsequent removal of oxygen would cause full reduction. Thus, the shift observed upon adding CO (O_2:CO - O_2:N_2) and the spectral shift observed upon changing from oxygen to nitrogen in the presence of CO (N_2:CO - O_2:CO) should sum to the total shift observed upon changing

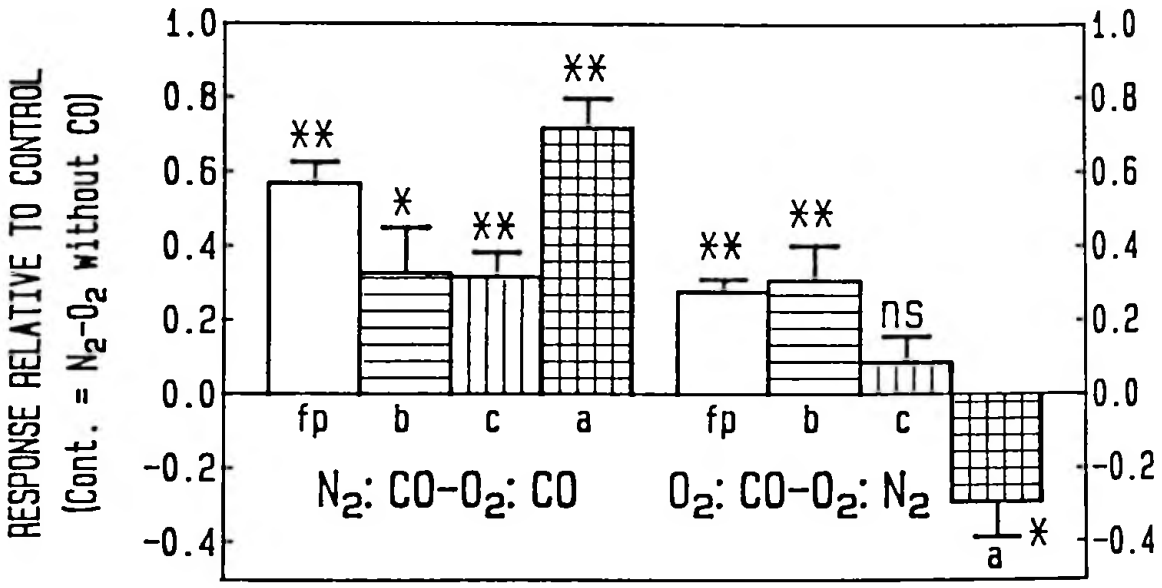

Figure 3. Summary of cytochrome responses to carbon monoxide, relative to response observed in that tissue without CO. Mean ±SE for 12 tissues. If CO had no effect, its addition under aerobic conditions should produce no change (ratio = 0). As shown on the right, the changes are significant (* = P<0.05, ** = P<0.01) except for cytochrome c. If CO completely blocked oxygen interactions, there should be no change between N_2:CO and O_2:CO, and this ratio should be zero. Again, the differences are all significant.

from oxygen to nitrogen in the absence of CO (N_2:N_2 - O_2:N_2). Examination of Figure 3 shows that this is not the case; the left and right sets do not sum to 1.0. Flavoprotein comes closest, with a sum of 0.87 ±0.05, which is still significantly different from 1.0. Cytochrome c shows the lowest total (0.41 ±0.08) with cytochrome a (0.46 ±0.11) at about the same level; cytochrome b is intermediate at 0.76 ±0.15. It may be important that the discrepancy is greatest for components toward the oxygen end of the chain, but no explanation is presently available for this effect.

These data taken with the previous observation of azide-insensitive cytochrome oxidation (but a sensitive cytochrome oxidase) suggest that an alternate route for oxygen reaction with this tissue may exist. Moreover, this route is capable of providing energy for acid secretion, unlike the "uncoupled" inhibitor-insensitive respiration in other tissues. This may relate to the hypothesis that gastric acid secretion is not ATP-driven.

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