INHIBITOR INSENSITIVITY OF OXYGEN CONSUMPTION OF CELLS FROM RAJA ERINACEA GASTRIC MUCOSA

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Acid secretion by the gastric mucosa of the little skate is an aerobic process. Since this tissue contains an apparently normal cytochrome system (Kidder & Kidder, Bull. MDIBL 25:40, 1985) the elimination of cytochrome oxidase activity by inhibitors should mimic the effect of anoxia. However, acid secretion is not abolished by a high CO/O_2 ratio (Kidder & Kidder, Bull. MDIBL 26:43, 1986), tissue oxygen uptake is relatively insensitive to azide (Kidder & Miller, Bull. MDIBL 27:106, 1987) and much of the cytochrome system continues to react with O_2 under these conditions. An alternate cytochrome oxidase is suspected which supplies energy for gastric acid secretion. Since oxygen consumption of the chambered tissue is diffusion-limited, which requires maintaining high O_2 concentrations, a cell suspension was prepared from the tissue for the present experiments.

The gastric mucosa was stripped of its heavy muscle layer, pinned mucosal side up on a parafin surface and exposed to elasmobranch Ringer's containing 1.75 mg/ml pronase (133 units/ml at 40°) for 3.5 hours at 30° in a shaking incubator under 95% O_2/5% CO_2. The cell suspension was centrifuged, washed and resuspended in a phosphate-buffered elasmobranch Ringer's with 20 mM glucose and 10 mM beta-hydroxybutyrate. The yield was 248 $\pm$ 35 x 10^6 cells per preparation by hemocytometer (N=33) of which 95.6 $\pm$ 0.5 % excluded eosine, in a total volume between 1 and 4 ml. Oxygen uptake was measured at 20° in an Instek oxygen electrode system, using 50-400 ul of cell suspension in a 600 ul chamber. Azide (N_3^-) and cyanide (CN^-) were added in volumes of 2 to 20 ul; oxygen uptake was determined from the slope of the recording of [O_2] vs. time, corrected for the dilution by inhibitor volume, and expressed as Q in ul $O_2/10^6$ cells·hr. The apparant Km for O_2 could be determined from the [O_2] at which Q was half of Q_{max}. Values are mean $\pm$ SE for (N) determinations; 9 tissues were used for each of the N_3^- and CN^- series, and 11 tissues for CO experiments.

There was considerable variability in Q between preparations. For the N_3^- series, the uninhibited Q was 0.704 $\pm$ 0.034 (67), while in the later CN^- series, the control Q was 0.586 $\pm$ 0.039 (62), a statistically significant ($P<0.01$) difference. To remove variability, each inhibited value was divided by the control portion of the same run; the resulting data are shown as Figure 1. For both inhibitors, there is a small inhibition at low concentrations, but a large fraction of Q remains at high con-

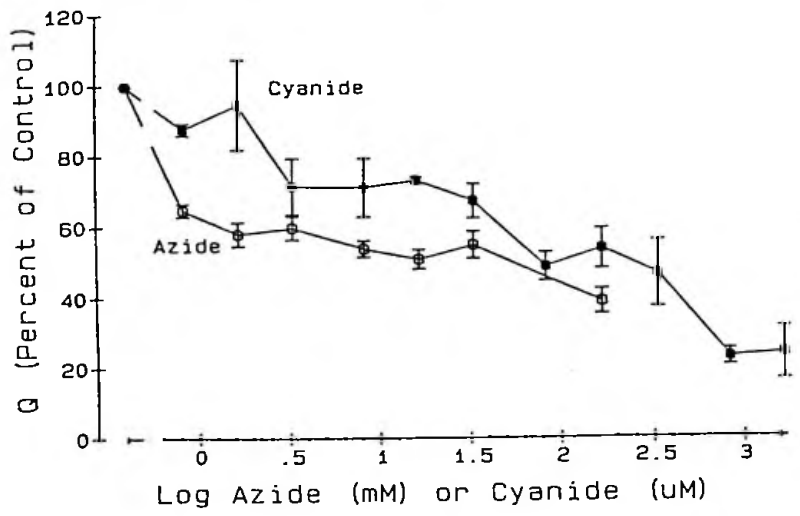

Figure 1 Azide and cyanide on Q

centrations, as was previously observed with the isolated tissue preparation. All values are different from both 100% and zero ($P<0.01$) except that the value for 1.67 uM CN^- (94.8 $\pm$ 12.8% of control) does not differ from no inhibition.

Noncompetitive inhibitors are not expected to change the Km of an enzyme. The effect of N_3^- and CN^- on the apparant Km for oxygen is shown in Figure 2. While N_3^- has no significant effect on Km, CN^- markedly and significantly increases Km at concentrations above 10 uM. Either CN^- is competitive or a second oxidase with a higher Km is being unmasked.

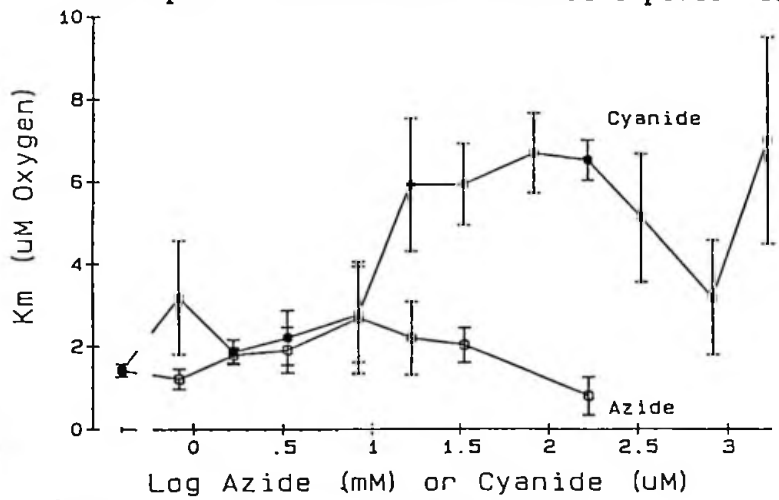

Figure 2 Azide and cyanide on Km

Carbon monoxide (CO) is a competitive inhibitor of cytochrome oxidase; we expect that $Q = Q_{max} \cdot K/([CO]/[O_2]+K)$, where K is the ratio of the affinities of the oxidase for O_2 and CO respectively. Starting with an O_2/CO mixture, Q should decrease as $[O_2]$ decreases, since [CO] does not change; $[CO]/[O_2]$ is calculated from the measured $[O_2]$ and the initial [CO]. As seen in Figure 3, the isolated gastric cells (solid points) are best fit by $30<K<70$, while yeast cells (solid +'s) have $5<K<10$ appropriate to cytochrome oxidase. Strong light, which photodissociates the cytochrome oxidase-CO complex, increases the apparant K of gastric cells (open circles) at high $[CO]/[O_2]$, as it does with yeast (not shown). The "Km" with light decreases from 7.73 $\pm$ 0.82 to 2.62 $\pm$ 0.42 uM (N=22), but not to the CO-free level of 1.42 $\pm$ 0.16 uM (N=30).

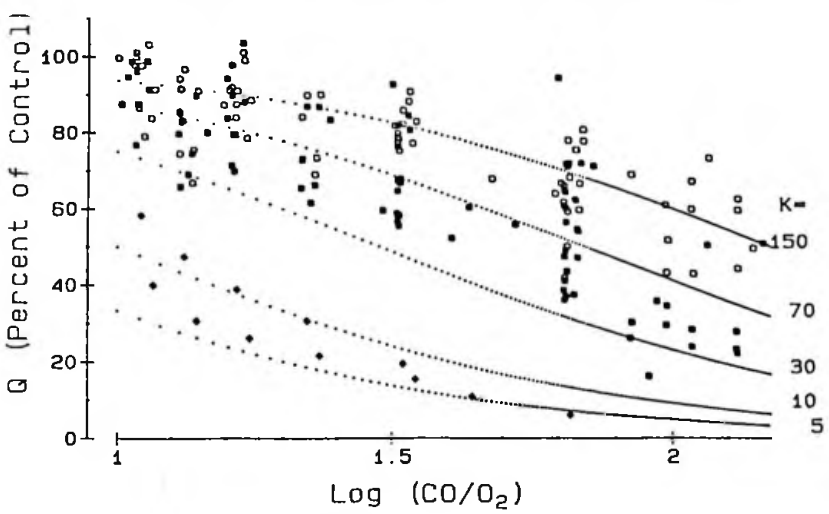

Figure 3 Effect of CO on Q

The cells have a respiration which is insensitive to N_3^-, CN^- and CO which is capable of 60 to 80% of control Q. This alternate oxidase may have a higher Km for O_2 than cytochrome oxidase, and its inhibition by CO is photoreversible. The cells also possess a conventional (sensitive) cytochrome oxidase, as shown by the inhibition of 20 to 40% of respiration at very low inhibitor concentrations. These findings are consistent with the spectrophotometric studies, and may indicate that gastric acid secretion is driven by a cytochrome system using the insensitive oxidase.

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