Effect of Cyanide on Oxygen Tension-Dependent Mechanical Tension in Rabbit Aorta

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SUMMARY We determined relationships between oxygen uptake and mechanical tension in isolated strips of rabbit aorta treated with various agonists; organ bath PO₂ was varied over the range 4-350 mm Hg, or cyanide (CN) was added to the organ bath in concentrations of from $8 \times 10^{-8}$ to $3 \times 10^{-5}$ M. Oxygen tension-dependent mechanical tension changes were similar during contractions caused by norepinephrine and angiotensin. During norepinephrine contractions, relaxations resulting from CN were much smaller than those resulting from decreases in organ bath PO₂ at equivalent rates of oxygen uptake. This effect could not be explained by nonspecific CN effects, which caused relaxation of mechanical tension. The threshold fall in oxygen uptake rate ($VO₂$) for a fall in mechanical tension was larger with graded CN than with graded hypoxia. Under conditions in which measurable oxygen uptakes by the strips were completely inhibited with CN or maximally inhibited with antimycin A, and tissue PO₂ was expected to be equilibrated or nearly equilibrated with organ bath PO₂, mechanical tension changes were seen with changes in PO₂ over the range 4-80 mm Hg. These data are consistent with a postulate that an O₂ sensor other than cytochrome oxidase is involved in the mechanism of oxygen tension-dependent mechanical tension, although inhibition of the respiratory chain may explain some oxygen tension-dependent mechanical tension. During K⁺ contractions, hypoxic decreases in mechanical tension were much smaller than those seen during norepinephrine or angiotensin contractions. This finding does not give information about the oxygen sensor involved, but the K⁺ contraction preparation is useful in control experiments. The finding that there was no difference in mechanical tension-oxygen uptake plots with CN and with hypoxia during K⁺ contractions, and with CN in norepinephrine contractions, suggests that effects of CN on tension may result from inhibition of the respiratory chain. During K⁺ contractions, hypoxic relaxations during norepinephrine contractions may be initiated by a mechanism independent of respiratory chain activity. Circ Res 44: 368-378, 1979

IT HAS BEEN SHOWN that "active" mechanical tension in rabbit aorta and other arterial smooth muscles is sensitive to oxygen tension changes (Grubb and Coburn, 1976; Carrier et al., 1964; Detar and Bohr, 1968; Fay, 1971; Pittman and Duling, 1973; Smith and Vane, 1966). There is some evidence obtained for the neonatal guinea pig ductus arteriosus (Fay, 1971; Fay and Jobsis, 1972), and based on measurements of $a_i/a_o$ spectra, carbon monoxide photodissociation, and other approaches, that cytochrome oxidase is the $O_2$ sensor involved in this phenomenon. In the present study, we attempted, using rabbit aorta, to obtain further evidence in support of or against the cytochrome oxidase sensor hypothesis.

The "critical" organ bath PO₂ (OBO₂) for an effect on mechanical tension in vascular smooth muscle is dependent on the thickness of the preparation and on other factors (Pittman and Duling, 1973). For oxygen to limit mitochondrial electron chain transport in a hypoxic core of a muscle strip, the PO₂ should fall as low as 0.05 mm Hg (Chance et al., 1977), and the PO₂ gradient from outside to hypoxic core, a diffusion distance of 60-100 μm in most vascular smooth muscle strips studied to date, would have to be as high as 200-300 mm Hg. Pittman and Duling (1973) made assumptions about diffusion geometry, $O_2$ diffusion coefficient, and oxygen uptake, in an attempt to compute the hypoxic core PO₂ at the critical OBO₂ (OBO₂ below which relaxations occurred), and concluded that it is possible that the core PO₂ may be low enough to limit cytochrome oxidase function. Fay et al. (1977) have measured core PO₂ in the ductus arteriosus with a polarographic electrode and concluded that PO₂ may be low enough to limit respiratory chain function; however, the polarographic electrode is not nearly capable of measuring PO₂ levels as low as the critical PO₂ of the cytochrome oxidase. It is possible that cytochrome oxidase has a higher $K_m$ in vivo than that measured in vitro (Jobsis, 1977).

Cytochrome oxidase does exhibit graded sensitiv-
ity to tissue $P_O_2$ in the intact brain (Jobsis, 1977), but it is not clear how this could be transduced to contractile proteins. It is possible that decreases in ATP concentration can influence tension, since biochemical studies show that ATP effects on myosin ATPase and disaggregation of actomyosin are qualitatively altered by changes in ATP concentration (Finlayson et al., 1969; Lymn and Taylor, 1970). However, there is considerable doubt about the status of overall energy production in vascular smooth muscle strips in an organ bath with decreases in OBPO2. Peterson and Paul (1974) found that energy production in mesenteric vein, computed from measurements of oxygen uptake ($V_O_2$) and lactate efflux, appeared not to change with acute, nearly complete removal of oxygen from the bathing solution. Needleman (Needleman and Blehem, 1970) found no change in ATP content of rabbit aorta during anoxia, although creatine phosphate levels decreased significantly. Other workers have measured rather small decreases in [ATP] in various vascular tissues during anoxia (Lundholm and Mohme-Lundholm, 1963, 1965; Shibata and Briggs, 1967; Van Horn et al., 1976; Namm and Zucker, 1973). It is of course possible that there is a fall in a high energy compound in a small cellular compartment that would not be detected in these types of experiments. It is clear that, in rabbit aorta, there are sufficient energy stores during complete anoxia for contraction to occur (Lundholm and Mohme-Lundholm, 1963, 1965; Shibata and Briggs, 1967).

Studies of the mechanism of $O_2$-sensitive mechanical tension ($MT_{O_2}$) in isolated smooth muscle are complicated by $P_O_2$ gradients in tissue and the possibility that effects of hypoxia on a very small portion of tissue in the tissue core could influence mechanical tension in smooth muscle cells on the periphery. Biochemical measurements at OBPO2 slightly below the critical level are not likely to give definitive data, due to the relatively small number of cells in the tissue core that are hypoxic. At a very low OBPO2, or during anoxia, demonstration of decreased energy production does not prove that mechanical events during hypoxia are initiated by the same oxygen-sensitive reactions.

In the present study we used cyanide (CN) and other metabolic inhibitors to investigate the possibility that cytochrome oxidase is the oxygen tension sensor. Oxygen uptake-mechanical tension relationships were determined at varying values of OBPO2 and with graded CN concentrations, and we looked for $O_2$-sensitive mechanical tension during inhibition of the respiratory chain. Metabolic inhibitors have been used previously in studying the phenomenon of oxygen tension-sensitive mechanical tension in vascular tissue (Fay, 1971), and it is known that these agents cause relaxations, but there exist no quantitative data or studies of possible nonspecific effects.

**Methods**

Male rabbits weighing 4-5 pounds were killed by cervical dislocation and exsanguination. The abdominal aorta was removed and the adventitia dissected. Transverse strips, $3 \times 11$ mm, were cut, varying in thickness from 0.17 to 0.23 mm. The strips were mounted in an organ bath similar to that described by Kroeger and Stephens (1971), which allowed simultaneous measurement of oxygen uptake and isometric tension. The organ bath had a volume of 1.4 ml and was controlled at 40° ± 0.1°C. Because of the low oxygen uptake of rabbit aorta, it was necessary to mount four strips. The bottom ends of the strips were tied to a bar at the bottom of the bath, and a string leading from the top ends of the strips was connected to an isometric tension transducer (model FT03C; Grass Instruments Co.). The strips did not touch each other, and care was taken to keep resting lengths uniform. All studies were performed with total resting tension on the four strips adjusted to 2 g (0.5 g/strip); this gave a length of 90-100% of $L_{max}$. The strips were equilibrated in the apparatus for 60 minutes before measurements were made. The organ bath was irrigated with Krebs' solution at a rate of 0.05-0.1 ml/min and constantly stirred with a bar magnet.

The $P_O_2$ in the organ bath was determined with an oxygen electrode (Yellow Springs Instruments, model 53). This instrument was calibrated by gasping with several different gases with different oxygen tensions, and with dithionite, and was found to be linear within ±3%. The rate of oxygen uptake ($V_O_2$) of the strips was determined by closing the top of the organ bath and measuring the rate of fall in $P_O_2$, as illustrated in Figure 1. Correction factors for electrode $O_2$ consumption and bath leakage were determined before the strips were mounted and at the end of each experiment. Correction factors at OBPO2 = 150 mm Hg were approximately 10% of total $V_O_2$, and 20% of total $V_O_2$ at 75 or 200 mm Hg. $V_O_2$ at nearly zero OBPO2 could be determined to within ±20 μl/(g x hr).

**Figure 1** Measurement of oxygen uptake. Tracing shows fall in $P_O_2$ with time in the closed organ bath containing four strips of rabbit aorta. Note that detectable oxygen uptake is abolished following injection of 1.5 mM NaCN.
The organ bath could be opened or closed without altering isometric tension. The bathing solution of the open organ bath was constantly gassed with gas mixtures. The flow rates of gases from two tanks (97% O₂ and 3% CO₂, and 97% N₂ and 3% CO₂) leading to the organ bath could be altered without changing Pco₂ or total gas flow. The closed OBPO₂ was changed by injection of warmed solutions of known Pco₂ at constant pH. In experiments with carbon monoxide (CO) we used tanks containing either 72% CO, 25% N₂, and 3% CO₂, or 72% CO, 25% O₂, and 3% CO₂. The percent CO₂ was matched in different tanks to within 0.04% and the pH in the Krebs' solution equilibrated with these gases varied less than 0.01 pH; on changing OBPO₂ from 0% to 100% O₂. With N₂-CO₂ gassing, OBPO₂ fell to levels of 4-7 mm Hg.

To demonstrate effects of OBPO₂ on tension in rabbit aorta it is necessary to generate active tension; previous investigations have used norepinephrine so that isolated rabbit aorta has a tension analogous to the in vivo situation in which tension primarily results from sympathetic adrenergic "input." We studied oxygen tension-sensitive mechanical tension (MT₄₀₀) during application of angiotensin, elevated [K⁺], and graded norepinephrine concentrations, so that we could evaluate the possibility that the reaction of norepinephrine with the tissue had an oxygen tension-sensitive mechanism.

In most experiments, our primary goal was to compare mechanical tension and Vₐₒ₂. Although Vₐₒ₂ and mechanical tension could be measured simultaneously in the closed chamber, better mechanical records were obtained with the organ bath open. The preparation was stable during multiple runs, and we changed the order of runs in different experiments.

Solution pH was measured with Radiometer or Corning pH meters. In some experiments a thermometer was inserted into the organ bath. Modified Krebs' solution had the following composition (in mM): Na⁺, 137; K⁺, 5.9; Mg²⁺, 1.2; Ca²⁺, 2.5; Cl⁻, 134; H₂PO₄⁻, 1.2; HCO₃⁻, 15.4; glucose, 11.5. The pH of this solution when equilibrated with 3% CO₂ was 7.33-7.38. In experiments in which OBPO₂ was altered (Vₐₒ₂ was not determined in this study), Figure 3 shows the relationship of OBPO₂ and Vₐₒ₂, and Figure 4 shows the relationship of Vₐₒ₂ and mechanical tension. Falls in active tension following injection of norepinephrine concentrations giv-
seen during norepinephrine contractions.

During 59 mM K\textsuperscript+ contractions, changes in mechanical tension resulting from changes in OBPO\textsubscript{2} were smaller than those seen during norepinephrine contractions (Fig. 5). The relationships of OBPO\textsubscript{2} to \(V_02\), however, were not different with K\textsuperscript+ and norepinephrine contractions (Fig. 3). Pretreatment with propranolol (3.8 \(\mu\)M) or phenoxybenzamine (4.3 \(\mu\)M) did not change \(M_{TAP}\) during K\textsuperscript+ contractions.

**CN Studies**

Effects of CN on \(V_02\) and mechanical tension, and the relationship of OBPO\textsubscript{2} to \(V_02\) and mechanical tension, were determined in two different types of experiments: (1) incremental CN injections in which CN was injected at a given OBPO\textsubscript{2}, and, after steady state effects on mechanical tension and \(V_02\) were determined, another injection was performed (this sequence was repeated several times at ranges of [CN] from 0.05 to about 2 mM); (2) single CN injections in which, after steady state effects were determined, the drug was washed out, the preparation allowed to recover for 1 hour, and the sequence repeated at another [CN] or OBPO\textsubscript{2}. The preparation was stable in either type of experiment after multiple runs, as judged by the \(V_02\) measurements and results in duplicate runs. Figure 6 shows effects of varying [CN] on \(V_02\) at OBPO\textsubscript{2} = 250 mm Hg, for which 50% inhibition occurred at [CN] of 0.08-0.11 mM. There was no difference with 59 mM K\textsuperscript+ or norepinephrine contractions. The rapid and persisting effect of CN on \(V_02\) is illustrated in Figure 1. CN injections at OBPO\textsubscript{2} above the critical OBPO\textsubscript{2} (>180 mm Hg) resulted in nearly exponential relaxations which were complete in 5-8 minutes. CN
injections at OBPO₂ below the critical PO₂, 50–180 mm Hg, resulted in a triphasic response consisting of an initial small relaxation lasting 20–30 seconds followed by a small contraction and a prolonged relaxation which was complete in 5–8 minutes. CN given at OBPO₂ < 7 mm Hg either had no effect on mechanical tension or caused a small relaxation.

Figure 4, which plots the decrease in norepinephrine mechanical tension vs. the fall in VO₂ due to increasing [CN], at OBPO₂ > 200 mm Hg, shows that for an equivalent fall in VO₂ due to CN there is a smaller decrease in mechanical tension than is seen in hypoxia experiments. During K⁺ contractions, CN injection caused falls in mechanical tension similar to those seen during hypoxia (Fig. 5).

CN might exert nonspecific effects (effects on tension unrelated to reaction with cytochrome aₐₐₐ) which would explain the small CN relaxations during norepinephrine contraction. As indicated above, CN (0.5–1 mM) injected during steady state relaxations caused by anoxia either had no effect on mechanical tension or caused an additional fall in tension. Administration of CN (after AA was given in amounts sufficient to depress VO₂ to less than 3% of control) caused relaxation (Fig. 7). A repeat of CN injection, after detectable VO₂ had been completely inhibited by CN, caused further relaxation of tension. These findings indicate that CN has an effect or effects that cause relaxation unrelated to inhibition of oxygen uptake. The triphasic mechanical tension response seen with CN under conditions in which OBPO₂ was in the range 50–180 mm Hg seems consistent with this conclusion. In these experiments in which core PO₂ was hypoxic at the time of CN injection, CN injection must have caused an increase in core PO₂ due to a fall in VO₂. Therefore the effect is complex, consisting of a CN relaxation plus an effect of increasing core PO₂ which is expected to cause an oxygen-dependent contraction.

With complete inhibition of detectable VO₂ with 1.5 mM CN, rabbit aortic strips still were capable of marked relaxation. This was demonstrated by showing that isoproterenol (2.6 mM) caused a relaxation that was comparable, in percent fall in active tension and rate of fall in tension, to data resulting from isoproterenol under control conditions. These data indicate that the energy state of the cell was sufficient to drive calcium pumps in organelles or plasma membrane, and that the tissue was not in rigor.

**Oxygen Sensitivity during CN**

These experiments were performed with the open organ bath, which allowed better mechanical records. OBPO₂ was adjusted over the range from 6–7 to ≥500 mm, and CN, 1.0–3.0 mM, was injected into
the organ bath fluid. After the CN relaxation was complete or nearly complete, OBPO$_2$ was either increased or decreased, and effects on mechanical tension were determined. In norepinephrine experiments, MT$_{\text{APo}2}$ always persisted, as illustrated in Figure 8. When OBPO$_2$ was returned to control, mechanical tension reversed. At the end of the runs, the organ bath was closed, and it was shown that VO$_2$ was nondetectable as long as the run was less than 12 minutes long. Presumably, with longer runs [CN] in the bath fell below levels required for complete inhibition of VO$_2$, or CN-insensitive respiration occurred. MT$_{\text{APo}2}$ was not detectable after CN injection during 59 mM K$^+$ contractions.

We could not compare MT$_{\text{APo}2}$ during CN with data obtained without CN under steady state conditions in open bath experiments, since CN relaxations were slow, as were relaxations occurring as a result of a decrease in OBPO$_2$, and CN was apparently lost from the organ bath too rapidly. Therefore, we have compared MT$_{\text{APo}2}$ during CN with MT$_{\text{APo}2}$ in the absence of CN, by comparing initial rates of changes of tension. This we defined as the highest rate of change seen over the 1st minute after tension change could be detected. This comparison is shown in Table 1. It should be pointed out that, in about half of the CN runs, OBPO$_2$ was altered prior to reaching CN steady state tension. In this case we computed initial change in mechanical tension as the difference in slope following change in OBPO$_2$. MT$_{\text{APo}2}$, on increasing OBPO$_2$ from low values are not shown in this table. In 18 norepinephrine experiments in which OBPO$_2$ was increased from values of 7–50 to 300–500 mm Hg in the presence of [CN], 1–3 mm, initial rate of increase in mechanical tension averaged 0.123 ± 0.05 (SEM) g/min, whereas under control conditions in the absence of CN, tension increased an average of 0.435 ± 0.09 g/min.

We also looked at MT$_{\text{APo}2}$ during exposure to CN with the organ bath closed. OBPO$_2$ was altered by injecting 0.5 ml of warmed Krebs’ solution containing norepinephrine. MT$_{\text{APo}2}$ were seen during CN treatment, and it was possible to obtain steady state data; however, we are not publishing these data because of the uncertainties regarding the matching of actual norepinephrine concentrations, small temperature changes, the possibility of diluting biologically active compounds (i.e., prostaglandin-like compounds), and possible mechanical effects of injecting a relative large quantity of fluid. Under “open” conditions in which the PO$_2$ of gas bubbling the organ bath was changed, it was reasonable to assume the only change in the muscle environment was PO$_2$.

It was not possible to match perfectly the PCO$_2$ in our N$_2$-CO$_2$ and O$_2$-CO$_2$ tanks, and change in OBPO$_2$ always caused some change in PCO$_2$ in pH or organ bath fluid. However, the possibility that changes in tension with changes in OBPO$_2$ in norepinephrine experiments could be related to extracellular pH changes was excluded by determining effects of small pH changes on tension, and by altering CO$_2$ tension in our tanks so that a given change in OBPO$_2$ was accompanied by the opposite mixtures were less than 0.02°C and could not explain tension changes on varying OBPO$_2$ in the presence of CN.

![Figure 8](http://circres.ahajournals.org/)

**Figure 8** Oxygen tension-sensitive mechanical tension following an injection of CN that completely inhibited detectable oxygen uptake. OBPO$_2$ was 228 mm Hg at the time of the HCN injection. At arrow indicating increase in OBPO$_2$. PO$_2$ gas bubbling the organ bath was decreased from 228 to 7 mm Hg. At arrow indicating PO$_2$ increase, PO$_2$ was returned to 228 mm Hg. In this run we also checked effects of small changes in PCO$_2$ in gas bubbling the organ bath. At the first arrow, PCO$_2$ increased from 2.95 to 3.20; at the second arrow, it decreased back to control level.

### Table 1 Oxygen-Dependent Mechanical Tension before and after CN

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Change in OBPO$_2$ (mm Hg)</th>
<th>Control MT$_{\text{AvPo}2}$, * (g/min)</th>
<th>After CN MT$_{\text{AvPo}2}$ (g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>326 to 12</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>532 to 7</td>
<td>0.10</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>270 to 14</td>
<td>0.30</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>250 to 21</td>
<td>0.15</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>260 to 21</td>
<td>0.15</td>
<td>1.0</td>
</tr>
<tr>
<td>6</td>
<td>326 to 7</td>
<td>0.09</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>281 to 14</td>
<td>0.35</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>304 to 3</td>
<td>0.08</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>280 to 3</td>
<td>0.25</td>
<td>3.0</td>
</tr>
<tr>
<td>10</td>
<td>395 to 28</td>
<td>0.28</td>
<td>1.5</td>
</tr>
<tr>
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<td>0.40</td>
<td>1.5</td>
</tr>
<tr>
<td>12</td>
<td>471 to 14</td>
<td>0.28</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>250 to 16</td>
<td>0.28</td>
<td>1.0</td>
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<tr>
<td>14</td>
<td>270 to 7</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>350 to 14</td>
<td>0.23</td>
<td>1.0</td>
</tr>
<tr>
<td>16</td>
<td>220 to 10</td>
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</tr>
<tr>
<td>17</td>
<td>230 to 7</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>18</td>
<td>240 to 6</td>
<td>0.23</td>
<td>1.0</td>
</tr>
<tr>
<td>19</td>
<td>300 to 10</td>
<td>0.30</td>
<td>1.5</td>
</tr>
<tr>
<td>20</td>
<td>250 to 7</td>
<td>0.30</td>
<td>1.5</td>
</tr>
<tr>
<td>21</td>
<td>240 to 7</td>
<td>0.24</td>
<td>3.0</td>
</tr>
</tbody>
</table>

| Mean          | 0.214                       | 0.074                                 |
| ± SEM         | 0.012                       | 0.017                                 |

* Initial rates of relaxation.
Experiments were performed to evaluate the possibility that CN inhibition of $\dot{V}O_2$ might be a function of $OBPO_2$. $OBPO_2$ was adjusted over the range 80-480 mm Hg, and $\dot{V}O_2$ was measured. CN was injected, giving a submaximal [CN], and $\dot{V}O_2$ was again determined. CN was then washed out, and the preparation was allowed to recover for 40 minutes. The sequence was repeated several times at different $OBPO_2$. Typical data, shown in Figure 9, indicate that $\dot{V}O_2$ after a constant [CN] is independent of $OBPO_2$.

**FIGURE 9** Effect of $OBPO_2$ on fall in $\dot{V}O_2$ resulting from injection of 0.8 mM NaCN. See text for details.

AA and Carbon Monoxide

AA (Estabrook, 1962) at a concentration of 0.5 ng/ml caused a decrease in $\dot{V}O_2$ to about 10% of control within 5 minutes, and maximal effects on $\dot{V}O_2$ were seen with 2-10 ng/ml. Maximal inhibition of $\dot{V}O_2$ resulted in falls to 2-4% of control, and further increases in AA had no additional effect on $\dot{V}O_2$. AA always caused a relaxation of norepinephrine tension (Fig. 8), and it was shown that AA injections given during maximal AA block of $\dot{V}O_2$ caused an additional relaxation. This may have been due to an effect of the solvent, $N,N$-dimethylformamide, since it was shown that, even at low concentrations, this solvent had a small effect on tension. During exposure to AA at $\dot{V}O_2 < 3\%$ of control, MT$_{TAP}=O$ was observed over the range of $OBPO_2$ from 7-80 mm Hg (norepinephrine contractions). Initial rates of change of mechanical tension on changing $OBPO_2$ during AA were similar to those observed during CN.

Norepinephrine mechanical tension and $\dot{V}O_2$ were resistant to very high Pco (Fig. 10). Switching from zero Pco, at a constant $OBPO_2 = 222$ mm Hg, to a Pco of 540 mm Hg resulted in a small relaxation and fall in $\dot{V}O_2$ to about 70% of control level. Administration of CO during CN block of $\dot{V}O_2$ (1.5-2 mM) resulted in relaxations. MT$_{TAP}=O$ persisted after CN plus CO. Altering $OBPO_2$ at constant Pco of approximately 540 mm Hg caused some blunting of the effects of decreasing $OBPO_2$ on mechanical tension for a given fall in $\dot{V}O_2$ (Fig. 4).

**FIGURE 10** Effect of CO (Pco, 540 mm Hg) on $\dot{V}O_2$ as a function of $OBPO_2$ during norepinephrine tension ($n = 5$). The Pco in gas bubbling the bath was increased, and after mechanical tension was steady, $\dot{V}O_2$ was determined. Organ bath Pco was then altered without changing the Pco$_2$, followed by repeat measurement of $\dot{V}O_2$. Control data were taken in the same preparations.

Discussion

In this study we confirmed findings of others that mechanical tension in an isolated strip of rabbit aorta can be markedly sensitive to organ bath Pco. This phenomenon is seen during submaximal to maximal norepinephrine contractions and during angiotensin contractions as well. These findings suggest that the mechanism is not related to endogenous norepinephrine release, uptake, or degradation, and that the O$_2$-sensitive component of contractions does not involve a mechanism unique to norepinephrine contractions. Although the O$_2$ sensor initiating O$_2$-sensitive mechanical tension could be located in intramural neural tissue, it does not involve cholinergic or adrenergic nerves, and more likely is located in the smooth muscle cell. With decreases in $OBPO_2$ below threshold level, falls in mechanical tension were always associated with decreases in $\dot{V}O_2$ (Fig. 4), as has been previously shown for other smooth muscles (Fay, 1971; Kroeger and Stephens, 1971). However, these data alone do not indicate whether tension fell because of O$_2$-limited aerobic energy production, or $\dot{V}O_2$ decreased due to reduced energy requirement, or $\dot{V}O_2$ fell independently of the fall in tension.

Our major approach in this study was to use respiratory chain inhibitors as a tool to examine the question whether cytochrome oxidase is the O$_2$ sensor involved in O$_2$-dependent mechanical tension. If an ideal inhibitor of the respiratory chain were available, it would be possible to perform definitive experiments answering the question whether MT$_{TAP}=O$ arises from a mechanism activated by a change in respiratory chain activity. Even if the mechanism of transduction of respiratory chain activity to contractile proteins were not understood, inactivation of the oxygen tension-sensing portion of the mechanism should inhibit MT$_{TAP}=O$. Unfortunately, an ideal respiratory chain inhibitor is not available. CN was used because it is primarily a gas...
at the pH used in these studies (Izatt et al., 1962), with a high diffusion coefficient that ensures even distribution in the tissue. This property is of paramount importance, since it is possible that a very few cells could trigger mechanical tension responses. A serious limitation to the use of CN in these studies is the nonspecificity of cellular effects of this agent. CN is known to bind to many different enzymes, including succinate dehydrogenase (Zanetti et al., 1973), xanthine oxidase and other molybdenum-containing iron sulfur flavoproteins (Massey and Edmondson, 1970), other heme proteins including cytochrome P-450 (Hiwatashi et al., 1975; Matsubara and Tochina, 1976), d-amino oxidase (Porter et al., 1972), superoxide dismutase, and carbonic anhydrase (Feeney and Brugen, 1973).

The other metabolic inhibitors used in this study have their limitations as well. AA is reportedly more specific for effects on the respiratory chain; however, there are no data, to our knowledge, of effects on other enzyme reactions. In addition, there is considerable doubt about the distribution of this lipid-soluble inhibitor in tissue. CO reacts with other hemoproteins, and in these experiments, as will be discussed below, did not completely inhibit Vo2 at a very high PCO.

Despite the nonspecificity of CN, we argue that it is possible to draw some conclusions from the results of the CN experiments. CN clearly inhibited the respiratory chain, as evidenced by the inhibition of all measurable oxygen uptake. The complete or nearly complete inhibition of Vo2 is of some interest in itself, since there are some reports of CN-insensitive respiration in tissue slices (Heymngen, 1938) which may reflect microsomal oxygen uptake. The tissue HCN concentration that inhibited Vo2 in our experiments was probably smaller than the value computed from CN introduced into the organ bath, due to CN binding and loss from the solution; therefore the smallest CN concentration that inhibited Vo2 is less than 6 x 10^{-5} M, and complete inhibition occurred at less than 10^{-3} M. CN effect in our experiments at [CN] less than 10^{-4} M may be more specific than at concentrations in excess of 10^{-3} M (Zanetti et al., 1973; Massey and Edmondson, 1970; Hiwatashi et al., 1975; Matsubara and Tochina, 1976; Porter et al., 1972).

One could argue that MTapO2 that occurred during CN inhibition of Vo2 was triggered in a few cells where cytochrome oxidase was not completely inhibited, and that cytochrome oxidase still could be the oxygen tension sensor initiating the signal that resulted in tension changes. However, the finding that MTapO2 persisted when [CN] was increased 3- to 4-fold more than required to completely inhibit measurable Vo2, and the high diffusion coefficient of HCN, make it unlikely that there could be respiratory chains in this tissue not completely inhibited by HCN. A basic question in interpreting these data is whether reaction of cytochrome oxidase with oxygen under conditions of complete inhibition of electron transport in the respiratory chain still could trigger a metabolic or physiological event that would result in a change in tension. No such reaction is known. Both binding of CN to cytochrome oxidase and inhibition of electron chain transport are influenced by the oxidation-reduction state and the resultant rate of electron flow (Van Buuren et al., 1972). It seems unlikely, however, that MTapO2 during CN could have been due to effects of changes in the oxidation-reduction state and consequent changes in CN inhibition, since oxygen uptake was not measurable in any of our experiments. CN and oxygen are not competitive in their reactions with cytochrome oxidase (Yonetani and Ray, 1965). This was confirmed by the finding that Vo2 at a given [CN] was independent of OBPO2. Therefore, the effects of increasing OBPO2 on mechanical tension during CN were not due to reduced CN inhibition of the respiratory chain electron transport. These findings suggest that the oxygen sensor initiating changes in mechanical tension is not cytochrome oxidase. Nonspecific CN effects should not influence this conclusion, since the argument is only that MTapO2 persists when the respiratory chain is completely inhibited. Similar findings with AA are also contrary to the postulate that respiratory chain inhibition is the initiating step in MTapO2. These findings also conflict with the possibility that a nonspecific effect of CN turned on a Po2-dependent mechanism that is not normally functioning in this tissue, or that MTapO2 could be related to a reversible nonspecific reaction of CN that altered mechanical tension and was competitive with O2. AA did not completely inhibit Vo2 even at very high concentrations, but this is expected, since mitochondrial studies have demonstrated an AA-insensitive oxygen uptake with endogenous substrate (Estabrook, 1962).

Our data (Table 1) indicate that initial rates of relaxation on lowering OBPO2 during CN inhibition of detectable Vo2 (norepinephrine tension) were about one-fourth of control initial rates of oxygen-dependent relaxations. However, control and CN MTapO2 are not strictly comparable, since the initial rate of relaxation is a function of active mechanical tension just prior to decreasing OBPO2. We therefore corrected our control relaxation data, using three experiments in which norepinephrine concentrations were smaller and gave mechanical tension similar to that found in the CN-treated preparation. This correction gave a figure for CN MTapO2 of 40-50% of control data. It appears that a significant portion of MTapO2 seen in control experiments is mediated by a mechanism that is resistant to CN.

Under conditions in which Vo2 is completely inhibited, tissue Po2 will equilibrate with OBPO2. The finding that changes in mechanical tension occurred during VO2 inhibition with CN or AA, as OBPO2 and tissue Po2 fell below 50-80 mm Hg, is very strong evidence that cytochrome oxidase was not the oxygen tension sensor, since the Km of
cytochrome oxidase is reported to be three orders of magnitude lower (Chance et al., 1977). The possibility that this phenomenon could be explained by the CN reaction with cytochrome oxidase altering the affinity for the reaction with oxygen is remote, since data shown in Figure 10 are at variance with interaction of CN and O2 effects on VO2. Furthermore, MTAP02 was seen during AA over the same OBPO2 range.

The VO2-mechanical tension plots obtained from CN runs (Figs. 4 and 5) could be influenced by nonspecific effects of CN. Indeed, by finding effects of CN under conditions where OBPO2 was nearly zero, during exposure to AA, or by giving CN increments in the presence of 1 to 2 mM CN, we show conclusively that CN has nonspecific effects on mechanical tension. However, it was shown that these nonspecific CN effects result in relaxation, and thus cannot explain the differences in VO2-tension relationships with graded [CN] vs. graded OBPO2 inhibition of VO2 during norepinephrine contractions. It should be stressed that this large difference in VO2-mechanical tension plots between the graded [CN] and graded hypoxia data occurred over the range of fall of VO2 from 100% to 70% of control. CN concentrations used over this range were 1 to 8 X 10^-5 M, concentrations at which effects of CN may be more specific, as discussed above. However, it is still possible that nonspecific effects of CN influenced VO2-mechanical tension data.

MTAP02 was markedly smaller during 59 mM K+ contractions compared to norepinephrine or angiotensin contractions. The explanation for this was not determined. OBPO2-V02 relationships and control VO2 did not differ (Fig. 3), suggesting that tissue PO2 gradients and core PO2 at a given OBPO2 were not different. Therefore, the smaller MTAP02 appears not to be due to different tissue PO2 in the two types of contractions. An obvious explanation might be that K+ contractions are more dependent on glycolytic energy, but, to the contrary, contractions appear to be less dependent on glycolytic energy (Needleman and Blehm, 1970; Lundholm and Mohme-Lundholm, 1963). There are other known differences in substrate requirements of K+ and catecholamine contractions (Godfraind, 1976). Also, there probably are differences in sources of activating Ca++. membrane potential-dependent processes (Goodman and Weiss, 1971; Altura and Altura, 1970), and other aspects of the biophysics of contraction and relaxation with K+ and with norepinephrine and angiotensin contractions. Hellstrand et al. (1977) have recently reported graded inhibition of surface membrane electrical activity in rat portal vein during hypoxia, and it is possible that in rabbit aorta there is a membrane potential-dependent step in the mechanism of MTAP02 that is inhibited by depolarization of the membrane with 59 mM K+. The smaller MTAP02 found with 59 mM K+ contractions does not argue per se for or against cytochrome a1/a3 being the O2 sensor involved in MTAP02. The K+ data, however, have value as controls for several of our experiments. The finding that MTAP02 is absent during CN with K+ contractions suggests that the persistence of MTAP02 during exposure to CN with norepinephrine contractions is neither an artifact nor due to turning-on of a PO2-sensitive mechanical tension mechanism that is not normally activated. The finding that VO2-mechanical tension relationships are not different with graded [CN] in both K+ and norepinephrine contractions and during hypoxia in K+ contractions may be coincidental. However, these data do suggest the possibility that the effect of CN on mechanical tension is mediated by inhibition of the respiratory chain, and that the greater relaxations seen at the same fall in VO2 in norepinephrine contractions due to hypoxia are mediated via another oxygen-sensing mechanism that is independent of the respiratory chain.

We note that the threshold decrease in VO2 for an effect on mechanical tension is larger with CN in both K+ and norepinephrine contractions, and with hypoxia in K+ contractions, than that seen with hypoxia in norepinephrine contractions. This finding suggests different mechanisms of sensing tissue PO2 in the norepinephrine-hypoxia experiments compared to the other experimental conditions.

The finding that drug-induced contractions or relaxations occurred when OBPO2 was less than 4–7 mm Hg, or during exposure to CN, seems consistent with the data of others showing that total tissue ATP does not fall markedly or at all in the absence of oxygen, and contradicts the respiratory chain hypothesis to explain MTAP02. Indeed, if high energy stores were depleted during anaerobiosis, one would expect the tissue to contract [due to release of calcium from intracellular depots or to inhibition of the plasma membrane Ca pump (Baker et al., 1971)], and/or to go into rigor. Neither of these results occurred in our experiments.

Studies with CO also seem to support participation of an oxygenase or receptor other than cytochrome oxidase. Carbon monoxide caused a decrease in mechanical tension and VO2. This could be considered evidence that electron transport in the respiratory chain is involved in MTAP02. However, CO is known to react with a variety of heme-proteins at the high partial pressures used here, and effects could involve mechanisms other than respiratory chain function. The finding that CO relaxed rabbit aorta under conditions in which CN had inhibited measurable VO2 suggests an effect on a hemoprotein other than cytochrome oxidase, as does the VO2-mechanical tension plot during CO as compared to that found in the absence of CO (Fig. 4).

The effects of CO on VO2 can be used to give gross estimations of mean tissue PO2, since CO and O2 react competitively with cytochrome oxidase (Keilin and Hartree, 1939; Wohlrab and Ogumnola,
1971), and because it is very likely that the effect of 
CO on VO₂ is due to reaction with cytochrome 
oxidase. In isolated mitochondria, a CO/O₂ ratio of 
5 to 10 is reported to be necessary for 50% VO₂ 
inhibition (Wohlrab and Ogumolins, 1971). Fifty 
percent inhibition of VO₂ occurred in our experi-
ments at an organ bath CO/O₂ ratio of about 10. 
Therefore, we deduce that mean tissue PO₂ may be 
only slightly less than OBPO₂, and that it is unlikely 
that core PO₂ has fallen below 0.05 mm Hg, even at 
OBPO₂ as low as 50 mm Hg. This is further evidence 
against cytochrome a₁a₂ as the O₂ sensor involved in 
initiating PO₂-sensitive mechanical tension.

We are surprised in light of previous work (Fay 
1971; Fay and Jobsis, 1972; Fay et al., 1977) that 
much of the data presented in this paper seems 
inconsistent with the hypothesis that the oxygen 
sensor involved in MT₃PO₂ is cytochrome oxidase. 
Our data are consistent with the possibility that 
some of the MT₃PO₂ could have been related to 
inhbition of the respiratory chain when VO₂ is 
hibited to levels below 50-70% of control. This 
follows from CN runs during K⁺ and norepinephrine 
contractions and hypoxic runs during K⁺ contrac-
tions.

If an oxygen tension sensor other than cyto-
brone oxidase is involved in MT₃PO₂ in rabbit 
aorta, we would postulate the following properties: 
It is not completely inhibited by CN or by AA. Its 
effective Kₐ is much higher than that of cytochrome 
oxidase, i.e., greater than 50 mm Hg. The VO₂ of 
reactions linked to this oxygenase or receptor is 
very small (and undetectable with our measure-
ments). To explain the fall in VO₂ that occurs with 
decreases in OBPO₂, it is necessary to postulate a 
decreased energy demand secondary to fall in ten-
sion. There is no information in our data that allows 
us to speculate about mechanisms by which signals 
from the oxygen tension sensor are transduced to 
the transduction process? 

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Acetylcholine Antagonism of the Electrophysiological Effects of Isoproterenol on Canine Cardiac Purkinje Fibers

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SUMMARY The purpose of these experiments was to determine whether or not acetylcholine modulated the electrophysiological effects of isoproterenol on canine cardiac Purkinje fibers. Conventional microelectrode techniques were used. Predictably, isoproterenol produced shortening of action potential duration; acetylcholine significantly blunted this effect of isoproterenol. Isoproterenol restored excitability to fibers exposed to 22 mM potassium solutions, and acetylcholine abolished this isoproterenol-restored excitability. Both of these antagonistic effects of acetylcholine were blocked by atropine. Acetylcholine alone did not affect action potential duration in polarized fibers or excitability in potassium-depolarized fibers. Furthermore, acetylcholine had no effect on the decrease in action potential duration induced by premature electrical stimulation or by acetylstrophanthidin administration, or on excitability of fibers exposed to a zero sodium, high calcium superfusant. These data demonstrate a direct cellular basis for cholinergic antagonism of the electrophysiological effects of β-adrenergic stimulation of canine cardiac specialized intraventricular conducting tissue. Circ Res 44: 378-383, 1979

CONTRARY TO long-established physiological concepts, several lines of evidence that developed during the past two decades indicate that cholinergic nerves innervate the ventricles of mammalian hearts (Higgins et al., 1973). The evidence includes histological demonstration of cholinergic nerve endings (Kent et al., 1974), histochemical localization of choline acetyltransferase [the enzyme that catalyzes the synthesis of acetylcholine (Jacobowitz et al., 1967; Roskoski et al., 1975)], and demonstration of the presence of acetylcholine in the ventricular myocardium (Brown, 1976). In addition to the evidence establishing the presence of cholinergic nerves in the ventricles, recent studies have shown that postsynaptic muscarinic cholinergic receptors exist on ventricular myocardial cells. By interacting with muscarinic cholinergic receptors, cholinergic agonists increase cardiac tissue cyclic guanosine 3′:5′-monophosphate concentrations (George et al., 1973; Watanabe and Besch, 1975a). Muscarinic cholinergic receptors on myocardial cells also have been directly demonstrated with ligand-binding assays using [3H]quinuclidinyl benzilate to label the receptors (Fields et al., 1978). Studies on the regulation of ventricular function by the autonomic nervous system support the conclusion that cholinergic nerves innervate the ventricles and, in addition, suggest that this innervation is functionally significant (De Geest et al., 1965; Daggett et al., 1967; Randall et al., 1968; Wildenthal et al., 1969). Stimulation of vagal nerves produced small reductions in contractility of dog hearts. When this stimulation was applied during simultaneous adrenergic stimulation, the negative inotropic effects were magnified (Levy et al., 1966; Dempsey and Cooper, 1969; Levy and Zieske, 1969; Levy, 1971, 1977). It thus appears that the cholinergic effects on ventricular contractile function are di-
Effect of cyanide on oxygen tension-dependent mechanical tension in rabbit aorta.
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