

Cyanide-induced Cytochrome a,a_3 Oxidation-Reduction Responses in Rat Brain In Vivo

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ABSTRACT The sensitivity of the brain to cyanide-induced histotoxic hypoxia and the protective effects of known cyanide antagonists, have been assessed in vivo by reflectance spectrophotometry. Cyanide-related changes in cytochrome a,a_3 (cytochrome c oxidase) oxidation-reduction (redox) state, tissue hemoglobin saturation, and local blood volume were continuously monitored in cerebral cortex of rats. Non-cumulative, dose-dependent inhibition of the in situ mitochondrial respiratory chain was evaluated directly by measuring increases in reduction levels of the terminal oxidase. These transient cytochrome a,a_3 reductions were accompanied by increases in regional cerebral hemoglobin saturation and blood volume. Cytochrome redox responses were not altered either in magnitude or kinetics by hyperoxia; however, the cyanide-cytochrome dose-response curve was greatly shifted to the right by pretreatment with sodium nitrite, and the recovery rate of cytochrome a,a_3 from cyanide-induced reduction was enhanced fourfold by pretreatment with sodium thiosulfate.

INTRODUCTION

Among the many known cellular effects of cyanide (1-6), inhibition of the mitochondrial respiratory chain has long been considered its predominant toxic effect (7). This in vitro action of cyanide, first observed by Keilin and Hartree (8) during absorption studies of cytochrome spectra in yeast and heart muscle preparations, defined cytochrome a_3 as the component of cytochrome a,a_3 (cytochrome c oxidase) that reacted with oxygen, carbon monoxide, and cyanide. Subsequent studies reported complete inhibition of cyto-

chrome oxidase in vitro with cyanide concentrations as low as 3×10^{-8} M (9), 50% decreases in cytochrome oxidase activity in rat brain homogenates after lethal intraperitoneal injections of sodium cyanide (10), and an inverse correlation between tissue cyanide levels and cytochrome oxidase activity in rabbits (11).

Prophylactic and therapeutic protection against death by cyanide poisoning has been reported for sodium nitrite, sodium thiosulfate, and oxygen (12-16), with enhancement of the protective effects of nitrite and thiosulfate by high oxygen partial pressures (16, 17). Nitrites oxidize ferrous hemoglobin to methemoglobin, which then competes with cytochrome c oxidase and other hemoproteins for cyanide, forming nontoxic cyanmethemoglobin (12). Thiosulfate distributes throughout total body water, binds to serum albumin, and functions as a sulfur donor for the enzyme rhodanese (thiosulfate sulfurtransferase), which catalyzes the transfer of a sulfur atom to cyanide, converting it to nontoxic thiocyanate (18-20). Rhodanese concentration is highest in hepatic mitochondria, however, because a large fraction of exogenously administered thiosulfate remains serum albumin bound and enhances serum rhodanese activity, serum has been proposed as a major in vivo site of cyanide detoxification (20). The means by which oxygen might potentiate cyanide antagonist activity is unknown, although a number of mechanisms, including activation of cyanide-insensitive respiration, oxygen displacement of cyanide from cytochrome oxidase by mass action, enhanced detoxification of cyanide by oxygen, and non-specific metabolic inhibitory effects of high PO_2 have all been proposed (14, 17).

Spectrophotometric evidence from mitochondrial and purified enzyme preparations has indicated that HCN binds with both the reduced and oxidized forms of cytochrome a_3 (21-24); however, the rate of interaction with oxidized cytochrome a_3 is two orders of magnitude slower than the reaction rate for reduced cytochrome a_3 and cyanide, leading others to conclude that the kinetically significant site of cyanide inter-

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ruption of mitochondrial electron transport is at the reduced cytochrome a_3 level (24).

Further progress in this field awaited development of dual wavelength spectrophotometry by Chance (25), making it possible to monitor cellular electron transport systems *in situ*. Subsequent technological advances by Jöbsis et al. (26) have allowed continuous, simultaneous, monitoring of cytochrome *c* oxidase redox state and changes in hemoglobin saturation and regional blood volume. Such technological application to living brain has shown that the redox state of cytochrome oxidase *in vivo* is labile to changes in tissue oxygenation (27, 28). This led us to speculate that the increased oxidation level of cytochrome a_3 in the presence of high oxygen partial pressures could decrease the rate of HCN binding to cytochrome a_3 during cyanide detoxification. A further objective was to define *in vivo* reversal of cyanide-induced cytochrome a_3 inhibition by known cyanide antagonists and possible mediation of their effects by oxygen.

METHODS

30 Adult male Sprague-Dawley rats (250–350 g) were used in the experiments. The animals were anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg *i.p.*) and tracheotomized. The inferior vena cava was cannulated via the femoral vein and both femoral arteries were cannulated for arterial blood gas sampling and continuous monitoring of arterial blood pressure. The animals were paralyzed with tubocurarine chloride (1.2 mg/kg *i.v.*) and upon cessation of spontaneous breathing were immediately placed on a positive pressure rodent respirator (Harvard Apparatus Co., Inc., S. Natick, MA, model 680) using a control gas mixture of 30% O_2 and 70% N_2 . Tidal volume and/or respiratory rate was adjusted to maintain normal P_{aCO_2} (35–40 mmHg) and $P_{aO_2} > 90$ mmHg; if not achieved, the animal was not studied. Rectal temperature was maintained at $37 \pm 1^\circ C$ by an external heating pad. The animal's head was placed in a stereotaxic head-holder and a longitudinal scalp incision was made exposing the dorsal skull. The skull was cleared to the periosteum, but maintained intact so as to leave intracranial pressure relationships undisturbed.

Changes in optical absorption characteristics of brain cytochrome *c* oxidase (cytochrome a_3) and hemoglobin saturation were continuously monitored through the translucent skull with a four-wavelength spectrophotometer. This instrument, used in the reflectance mode as described by Jöbsis et al. (26), allowed incident light to pass through four tunable monochromators, with existing sample and reference beams alternately passing down a thin fiber bundle for illumination of the skull and underlying cerebral cortex. The actual tissue penetration depth of the reflected light was not precisely known, but was probably less than full cortical thickness. A penetration estimate of 0.5 mm during reduced pyridine nucleotide fluorescence has been made in fully exposed tissue (29), but this estimate does not include light-scattering effects of bone or consider the dependence of penetration depth upon incident wavelength (30). Reflected light was collected with an internally reflecting clad glass rod coupled to the skull with an optical gel and shielded by an O-ring (Fig. 1). The intensity of this light was measured by a side window photomultiplier (Hamamatsu, R928) and

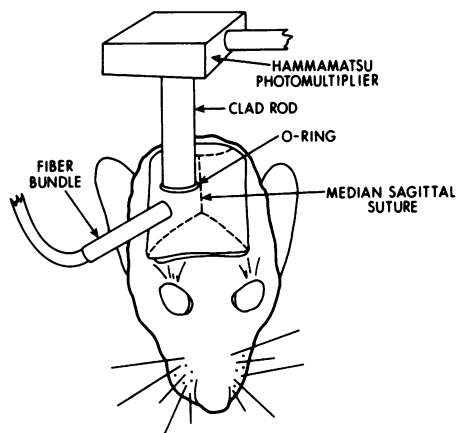


FIGURE 1 Reflectance spectrophotometry arrangement. Sample and reference light beams from tunable monochromators alternately passed down a 6.35 mm fiber bundle illuminating the skull and underlying cerebral cortex. Reflected light was collected in an internally clad glass rod coupled to the skull with optical gel and shielded by an O-ring.

the differences in intensity of sample and reference wavelengths were recorded on a multi-channel strip chart recorder (Beckman Instruments, Inc., Fullerton, CA, model R611).

Changes in tissue hemoglobin saturation were estimated by subtracting the hemoglobin isosbestic wavelength at 569 nm from the oxyhemoglobin absorption maximum at 577 nm. To compensate for blood volume changes that would produce changes in absorption at the 569 nm reference wavelength, this reference signal was held constant by feedback on the photomultiplier gain. This feedback signal was then used to follow relative changes in regional blood volume (26).

The optical absorption characteristics of cytochrome *c* oxidase depend upon the redox state of the enzyme. The difference spectrum (reduced-oxidized) shows an absorption maximum at ~603–605 nm predominantly derived from cytochrome *a* heme (31). Absorption changes at this 605-nm sample wavelength were compared with absorption changes at a reference wavelength of 620 nm. The 590-nm hemoglobin equibestic reference wavelength usually employed in this laboratory (26) could not be used in these cyanide studies because a prominent reduced cytochrome a_3 -CN absorption shoulder occurs at 585–590 nm (23). Consequently, the 605–620-nm pair measured primarily changes in the redox state of the cytochrome *a* heme moiety.

Changes in the oxygenation state of blood in tissue will also produce an optical signal at 605–620 nm, since this is not an equibestic pair for hemoglobin. The spectral interference of these regional hemoglobin-oxyhemoglobin transitions at the 605–620 cytochrome pair was predicted to be <12% of the optical density change at the hemoglobin pair (577–569 nm) because of the weak absorption differences between hemoglobin and oxyhemoglobin at 605 and 620 nm (32). Cyanide-induced histotoxic hypoxia shifts tissue steady state blood color towards increased oxyhemoglobin. This produces an increase in optical density at 605 relative to 620 nm that cannot be distinguished from cytochrome a_3 reduction. Therefore, the magnitude of cytochrome *a* reduction from cyanide will be overestimated by ~12% of the optical density change at the 577–569-nm oxyhemoglobin

wavelength pair. 620 nm has been previously used as a cytochrome reference wavelength in the presence of hemoglobin or myoglobin (33, 34).

Animals were divided into groups according to FI_{O_2} and treatment modality (Table I). After stabilization on normoxic or hyperoxic breathing gas, graded sublethal doses of fresh potassium cyanide (KCN, J. T. Baker Chemical Co., Phillipsburg, NJ, 2–4 mg/ml; maximum infusate volume 0.5 ml, 0.125–2.000 mg/kg) in normal saline were randomly administered intravenously over 1 min after control injections of equimolar KCl in normal saline were shown not to affect cerebral cytochrome a, a_3 redox level.

Spectrophotometric transients were allowed to return to base line before subsequent cyanide infusion. After the initial series of cyanide injections, the breathing gas was shifted to the alternate mixture or the animal was treated with either sodium nitrite ($NaNO_2$, 20 mg/kg i.v.) or sodium thiosulfate ($Na_2S_2O_3$, 500 mg/kg i.v.) infused over 5–10 min. Blood gases were again measured and the series of KCN doses was repeated. To establish maximum reduction levels of cerebral cytochrome c oxidase, all preparations were terminated with 100% N_2 or a lethal dose of KCN.

Venous blood samples (0.5 ml) were obtained before and 10 min after treatment with sodium nitrite to determine methemoglobin saturation. Duplicate sample measurements by modifications of the method of Evelyn and Malloy (36) showed that mean methemoglobin saturation was less than 1% before $NaNO_2$ treatment and $13 \pm 1.5\%$ (range 11–16%) after treatment.

RESULTS

Cyanide administration produced transient responses in all three optical signals (Fig. 2). Dose-dependent increases in optical density at 605 nm relative to 620

nm largely represent reduction of cytochrome a resulting from the electron-transport block by cytochrome a_3 -cyanide complex. These responses were accompanied by increases in oxyhemoglobin saturation and mild hypotension (Fig. 2). The nadir of the cyanide response (P_{max}) occurred at the peak oxyhemoglobin response and well after the nadir of the blood pressure response. Although hypotension might be expected to decrease cerebral perfusion, thereby contributing to the reduction of cytochrome c oxidase, blood pressure usually did not fall below the autoregulatory threshold and relative blood volume in the field increased. Additionally, equivalent degrees of transient hypotension induced by pentobarbital anesthesia did not produce cytochrome a, a_3 reduction.

Changes in cytochrome a, a_3 redox state were reported in two ways. In continuous tracings, changes were expressed as a percentage of full scale light intensity; zero being reference beam illumination of the cortex without sample beam illumination and 100% full scale being equal signals from reference and sample beams with the rat in a normoxic, unstressed condition before cyanide administration. Subsequently, the maximum response (P_{max}) to any given dose of cyanide was expressed as a percentage of the total labile signal determined by the absorption difference between this normoxic base line and the redox level at death (35). The position of each dose response curve was defined according to that cyanide dose required to achieve 50% maximum cytochrome c oxidase re-

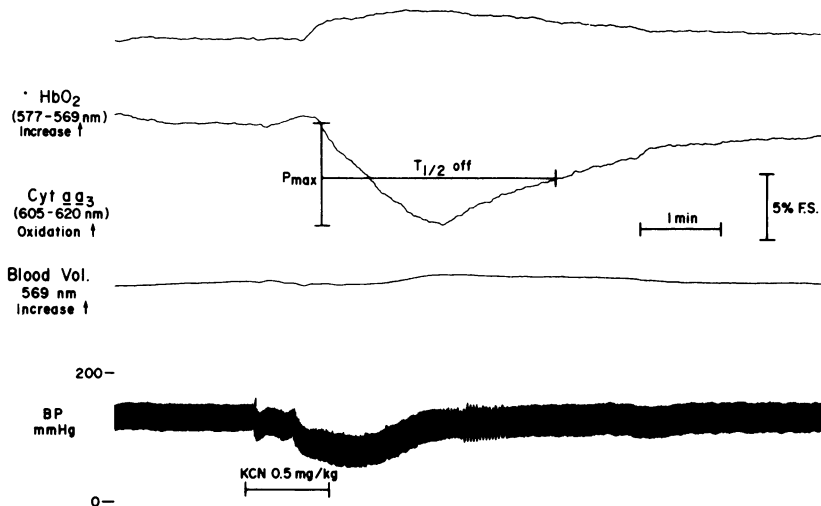


FIGURE 2 Continuous recording of metabolic and hemodynamic effects of KCN. Intravenous infusion of KCN produced transient increases in the reduction level of cytochrome c oxidase (cyt a, a_3) measured as absorbance difference at 605–620 nm. These cytochrome redox transients were associated with increases in hemoglobin saturation (577–569 nm) and regional blood volume (569 nm). The maximum absorbance change from base-line redox level (P_{max}) and time in min for 50% return to redox base-line ($t_{1/2}$ off) were recorded for each cyanide dose.

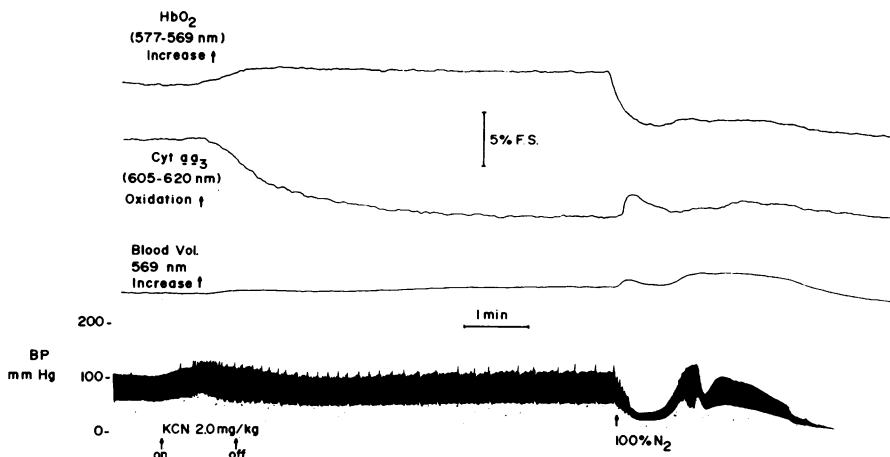


FIGURE 3 Maximum reduction of cytochrome oxidase by KCN. Intravenous KCN (2.0 mg/kg) produced full reduction of cytochrome oxidase as shown by no further change in absorbance at 605–620 nm with oxyhemoglobin desaturation during nitrogen death. Total labile cytochrome signal is the difference between resting normoxic baseline and death at 605–620 nm.

duction (K_D). The time from the start of a response until 50% return to base line ($t_{1/2}$ off) was also measured for each cyanide dose (Fig. 2). Recovery from respiratory chain inhibition was evaluated by least squares analysis of cyanide dose vs. $t_{1/2}$ off relationship.

A large dose of KCN (2.0 mg/kg) followed by nitrogen death produced the changes in oxyhemoglobin and cytochrome redox transients shown in Fig. 3. Large cyanide doses resulted in maximum reduction of cytochrome oxidase as shown by the lack of further reduction during oxyhemoglobin desaturation with 100% nitrogen. The large decrease in optical density at 577 relative to 569 nm during nitrogen hypoxia was associated with a small optical density change at 605–620 nm in the direction of cytochrome a,a_3 oxidation (See Fig. 3). Since cyanide followed by nitrogen hypoxia produces complete and final cytochrome a,a_3 reduction, these absorbance changes at 605 relative to 620 nm would provide an estimate of the contribution of hemoglobin to the cytochrome signal. In six such experiments, the optical density change in the cyanide reduced 605–620 nm wavelength pair during hemoglobin deoxygenation with nitrogen was $14 \pm 3\%$ of the optical density change at the 577–569 nm hemoglobin wavelength pair. This value was in good agreement with the 12% predicted spectral contribution of hemoglobin to the 605–620 nm pair.

Typical cyanide-cytochrome dose response curves for P_{max} during normoxia and hyperoxia are shown in Fig. 4. As summarized in Tables I and II, hyperoxia had no significant effect on the position of this curve (K_D , Group 1, Table I) or on the nearly linear dose dependent rate of recovery from the cyanide induced respiratory chain block (Slope, Group 1, Table II).

The effects of pretreatment with $Na_2S_2O_3$ on cyanide-induced cytochrome redox transients are shown in Figs. 5 and 6, respectively. The administration of $Na_2S_2O_3$ shifted the posttreatment dose-response curve slightly to the right (Fig. 6), and this small effect was not altered during hyperoxia (K_D , Groups 2 and 3, Table I). The major protective effect of pre-treatment with $Na_2S_2O_3$ was seen during the recovery of the cytochrome oxidase redox transient after cyanide administration (Fig. 5). $Na_2S_2O_3$ markedly decreased the slope of the KCN dose vs. $t_{1/2}$ off relationship indicating greatly accelerated detoxification of the cyanide (Slope, Group 2, Table II). No further effect was seen during hyperoxia (Slope, Group 3, Table II).

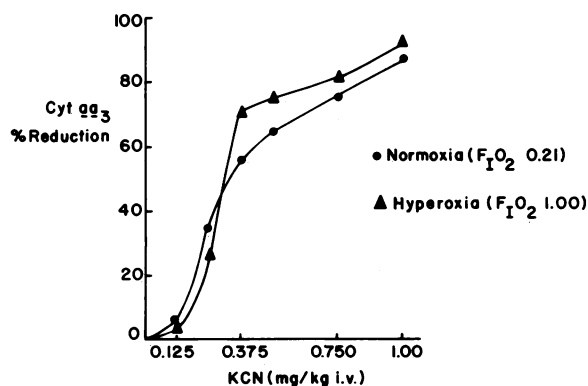


FIGURE 4 Cyanide-cytochrome dose-response curves: KCN vs. percentage reduction of cytochrome ($t_{1/2}$ a,a_3). Dose of KCN (milligram per kilogram, intravenous) was plotted against P_{max} (Fig. 2) expressed as a percentage of the total labile signal (Fig. 3). Hyperoxia had no effect on the position of the dose-response curves.

TABLE I
Effect of Oxygen and Cyanide Antagonists on Cyanide-induced Cytochrome *a,a*₃ Inhibition

	Pretreatment		Antagonist	Posttreatment		
	F _{IO₂}	K _D *		F _{IO₂}	K _D *	
Group 1 effect of hyperoxia	0.21	0.407±0.126	—	1.00	0.365±0.109	NS†
Group 2 effect of Na ₂ S ₂ O ₃	0.21	0.242±0.062	Sodium thiosulfate	0.21	0.589±0.300	P‡ < 0.01
Group 3 effect of Na ₂ S ₂ O ₃	1.00	0.313±0.086	Sodium thiosulfate	1.00	0.581±0.222	P‡ < 0.025
Group 4 effect of NaNO ₂	0.21	0.295±0.130	Sodium nitrite	0.21	1.235±0.213	P‡ < 0.0005
Group 5 effect of NaNO ₂	1.00	0.327±0.130	Sodium nitrite	1.00	1.408±0.350	P‡ < 0.0005

* K_D Mean±SD cyanide dose in mg/kg i.v. required to achieve 50% maximum cytochrome *a,a*₃ reduction.

† The *t* test, pretreatment vs. posttreatment groups.

Use of intravenous sodium nitrite to produce methemoglobin causes base-line changes in the three optical signals (Fig. 7). Oxidation of hemoglobin to methemoglobin increases optical density at 620 nm relative to 605 nm (32), resulting in a rising base line at the 605–620 nm wavelength pair. Approximately 60% more light is transmitted at 577 nm relative to 569 nm during conversion of hemoglobin to methemoglobin, causing an apparent desaturation of hemoglobin at 577–569 nm (Fig. 7). Physiological compensation also occurs because constant tissue oxygen consumption requires actual oxyhemoglobin delivery to the optical field to remain constant in spite of the methemoglobin, which does not carry oxygen. Consequently, hemoglobin saturation as measured at the 577–569-nm wavelength pair was only slightly affected by NaNO₂. A single dose of NaNO₂ greatly inhibited cyanide binding of cytochrome *a,a*₃ (Fig. 8), shifting the dose-response relationship to the right (Fig. 9). No further enhancement of this effect was seen in the presence

of hyperoxia (K_D, Groups 4 and 5, Table I). Redox recovery kinetics were only slightly enhanced following NaNO₂ treatment (Slope, Groups 4 and 5, Table II). It should be noted that administration of cyanide in the presence of methemoglobin creates an optical effect because of the conversion of methemoglobin to cyanomethemoglobin. This event decreases optical density twice as much at 620 nm compared with 605 nm (32), causing the 605–620 nm cytochrome pair to change in the direction of cytochrome *a,a*₃ reduction. The net effect is an overestimation of cytochrome *a,a*₃ reduction by cyanide, thereby underestimating the protective effect of methemoglobin.

DISCUSSION

Administration of oxygen would not a priori be expected to antagonize cyanide, since cellular oxygen utilization is already impaired and oxygen delivery to tissue decreases only when cardiac output falls. Fur-

TABLE II
Effect of Oxygen and Cyanide Antagonists on Cyanide Dose—*t* ½ off* Relationship

	Pretreatment		Antagonist	Posttreatment		
	F _{IO₂}	Slope†		F _{IO₂}	Slope†	
Group 1	0.21	7.12±3.10	—	1.00	6.70±3.00	NS
Group 2	0.21	8.08±2.00	Sodium thiosulfate	0.21	1.65±0.64	P‡ < 0.005
Group 3	1.00	6.67±1.57	Sodium thiosulfate	1.00	1.53±0.34	P‡ < 0.0005
Group 4	0.21	6.32±1.44	Sodium nitrite	0.21	4.19±1.10	P‡ < 0.010
Group 5	1.00	6.01±0.94	Sodium nitrite	1.00	4.26±1.06	P‡ < 0.010

* *t* ½ off is time in min from start of cyanide-induced cytochrome *a,a*₃ inhibition to 50% return to base-line redox state.

† Slope of least squares analysis of cyanide dose (milligrams per kilogram) against *t* ½ off (see Fig. 6).

‡ The *t* test, pretreatment vs. posttreatment groups.

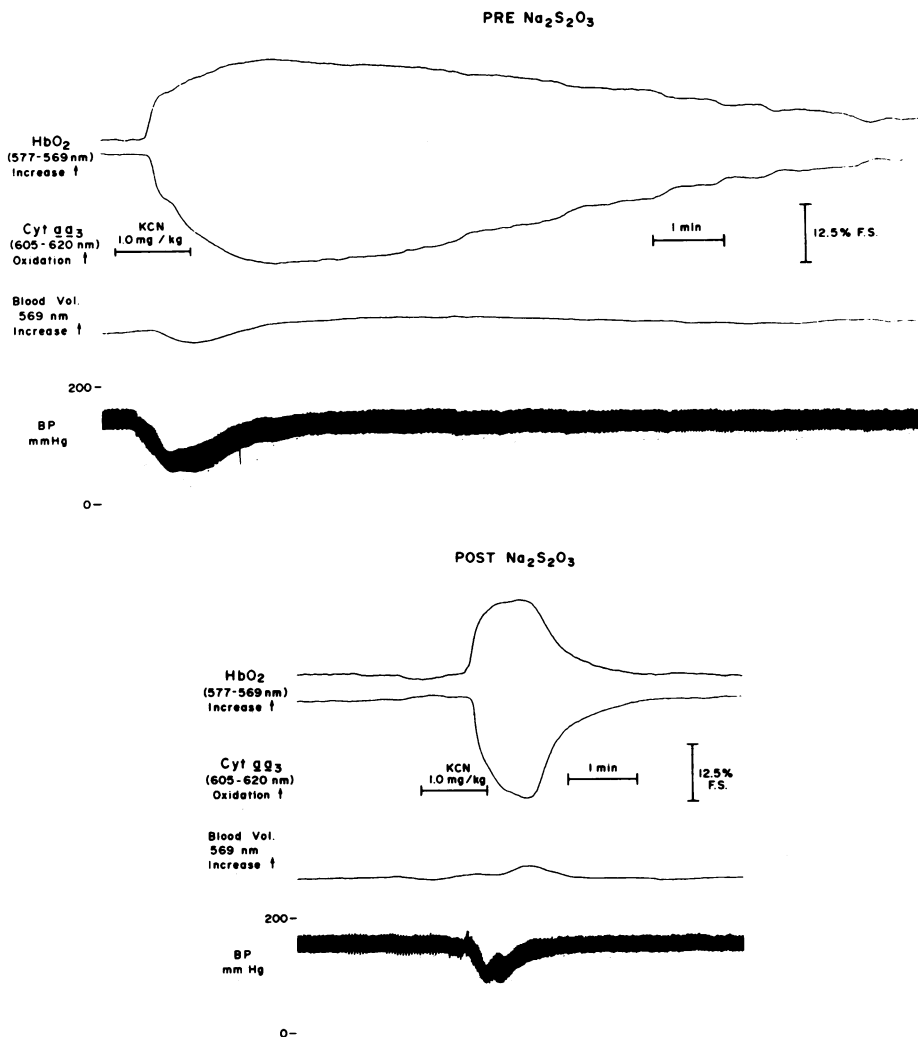


FIGURE 5 Effects of $\text{Na}_2\text{S}_2\text{O}_3$ on cyanide-induced cytochrome oxidase redox transients. Continuous recordings of KCN induced changes in cytochrome oxidase redox and hemodynamic transients before (top) and after (bottom) treatment with $\text{Na}_2\text{S}_2\text{O}_3$. $\text{Na}_2\text{S}_2\text{O}_3$ pretreatment resulted in rapid recovery from cyanide-induced cytochrome *c* oxidase inhibition.

thermore, under conditions of cyanide hypoxia, hemoglobin is fully saturated, cerebral blood flow increases, and arterialization of venous blood occurs (37). Using noninvasive methodology, we have shown non-cumulative, dose-dependent inhibition of the mitochondrial respiratory chain by sublethal doses of cyanide and have recorded accompanying increases in regional cerebral hemoglobin saturation and blood volume. The magnitude and kinetics of these metabolic and hemodynamic transients could not be appreciably modified by hyperoxia. The *in vivo* cytochrome *a,a*₃ recovery kinetics observed in this study are similar to those found by Schubert and Brill (38),

when, subsequent to KCN poisoning, they sequentially measured rat and mouse liver cytochrome oxidase activity *in vitro*. The apparent lack of a hyperoxic effect *in vivo* supports *in vitro* data showing that cyanide and oxygen are noncompetitive in purified preparations of cytochrome *c* oxidase (24) and that inhibition of oxygen uptake in isolated aortic smooth muscle by cyanide is independent of oxygen tension (39). Also, an oxygen effect on cyanide-mediated increases in reduced pyridine nucleotide fluorescence in the *in situ* rabbit renal cortex shown by Takano et al. (40) is not inconsistent with the results of the present study. During sublethal cyanide hypoxia, reduced pyridine nu-

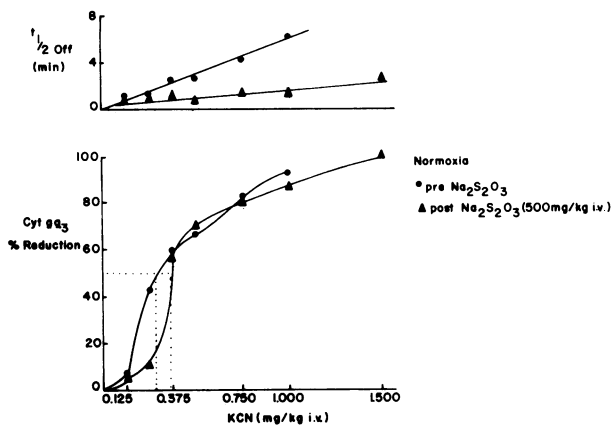


FIGURE 6 Effect of $\text{Na}_2\text{S}_2\text{O}_3$ treatment on cyanide-cytochrome dose-response curve. Lower plot was obtained as described in Fig. 4. Dotted lines show cyanide dose for 50% cytochrome oxidase reduction (K_D). Upper plot shows linear relationship between cyanide dose and time in min after KCN to 50% recovery of cytochrome redox transient ($t_{1/2}$ off, Fig. 2).

cleotides may be oxidized in some other way. Unblocked respiratory chains may "branch out" and oxidize CN-blocked chains (41) and it is possible that the multienzyme respiratory chain maintains some electron flow by a cushioning effect (42).

Although hyperoxia did not alter the cerebral metabolic responses to cyanide, these responses could be reproducibly modified in different ways by cyanide

antagonists. The primary effect of NaNO_2 -induced methemoglobinemia is a highly significant rightward shift of the KCN-cytochrome a, a_3 dose-response curve. This finding suggests competition for cyanide between ferric heme in methemoglobin and the cytochrome a_3 heme moiety, an interpretation consistent with the in vitro concept of a methemoglobin sink for cyanide (12). It must be realized that conversion of methemoglobin to cyanomethemoglobin affects our cytochrome a, a_3 redox state measurement by decreasing optical density more at 620 nm than at 605 nm. This causes overestimation of the extent of cytochrome a, a_3 reduction by cyanide in the presence of methemoglobin. Consequently, these data underestimate the cytochrome a, a_3 protective effect of methemoglobin. Slightly enhanced cytochrome a, a_3 redox state recovery from cyanide was also a feature of NaNO_2 pretreatment; however, because of the optical effects of forming cyanomethemoglobin from methemoglobin, this effect could be due to either more rapid relief of the electron transport block, conversion of cyanomethemoglobin back to methemoglobin, or both.

Sodium thiosulfate pretreatment exerts a major effect on recovery from cyanide-induced cytochrome a, a_3 reduction as shown by a fourfold decrease in the slope of the $t_{1/2}$ off-cyanide relationship to the control value. This rapid redox recovery rate contrasts with only a twofold increase in the intravenous cyanide dose necessary to produce half maximal reduction of cytochrome c oxidase after administration of thiosulfate.

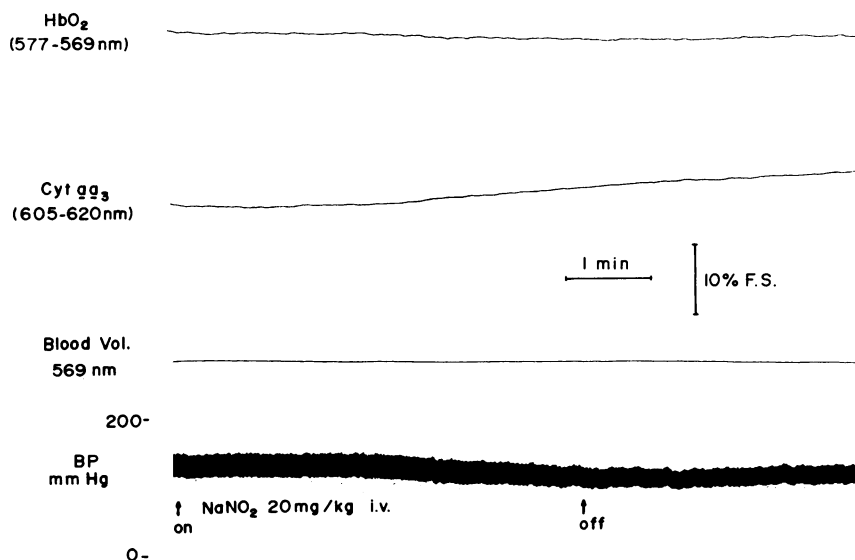


FIGURE 7 Formation of methemoglobin by sodium nitrite. Intravenous sodium nitrite produces a decrease in blood pressure and an apparent oxidation of cytochrome a, a_3 . The optical effect at the 605-620 nm wavelength pair is attributable to methemoglobin formation (see text).

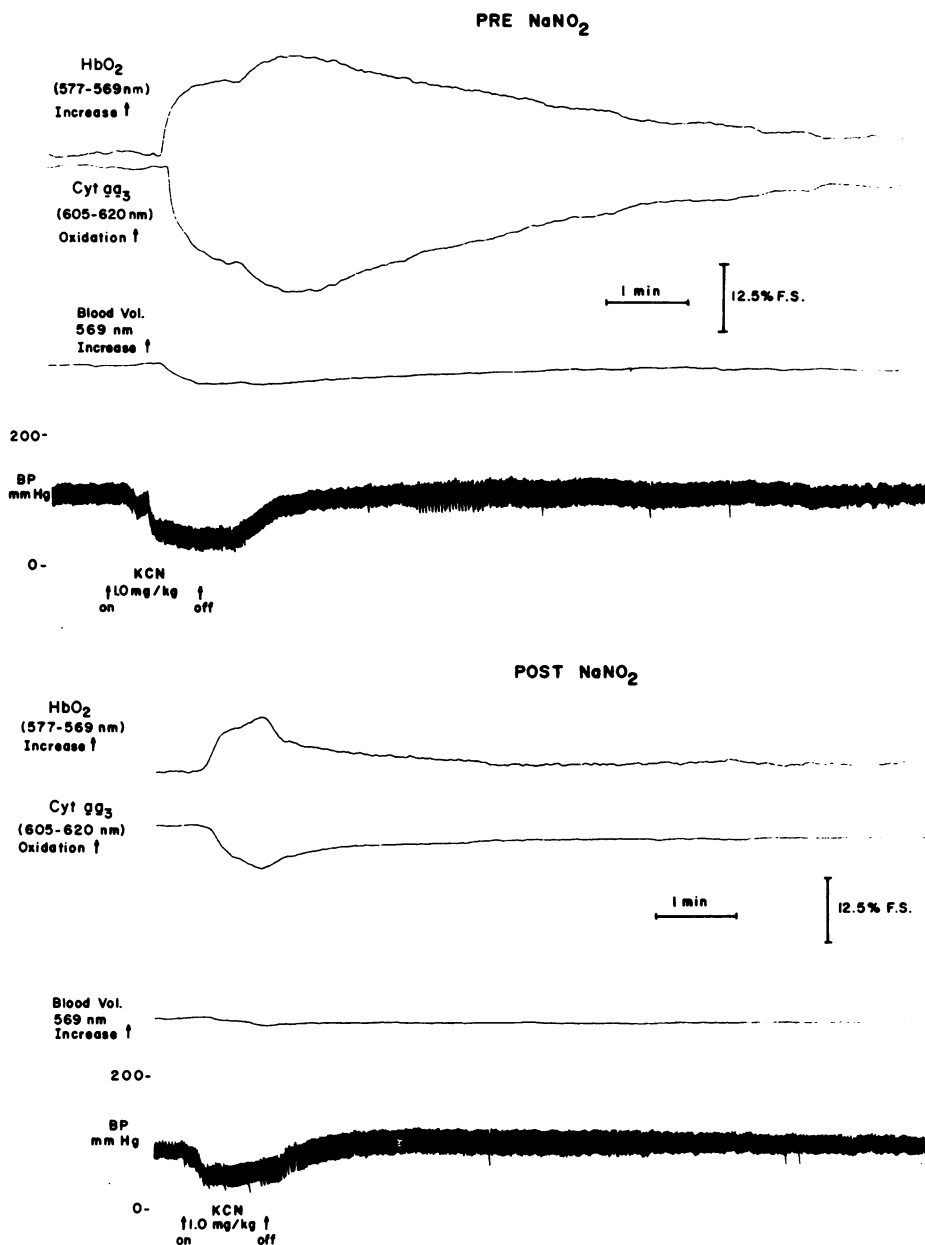


FIGURE 8 Effects of NaNO_2 on cyanide-induced cytochrome oxidase redox transients. Continuous recordings of KCN induced changes in cytochrome oxidase redox and hemodynamic transients before (*top*) and (*bottom*) treatment with NaNO_2 . NaNO_2 prevented cyanide-induced cytochrome *c* oxidase reduction.

Consequently, in the presence of thiosulfate, twice as much cyanide is metabolized by hepatic and serum rhodanese (20) during the period of cyanide-cytochrome interaction, while the rate of detoxification of already formed cytochrome-cyanide complex is enhanced fourfold. Such a discrepancy suggests that sig-

nificant thiosulfate protection occurs at the cellular level, even though brain rhodanese content is low (19) and little thiosulfate penetrates the inner mitochondrial membrane to reach thiosulfate sulfurtransferase (20).

Although we cannot now provide a cytochrome *c*

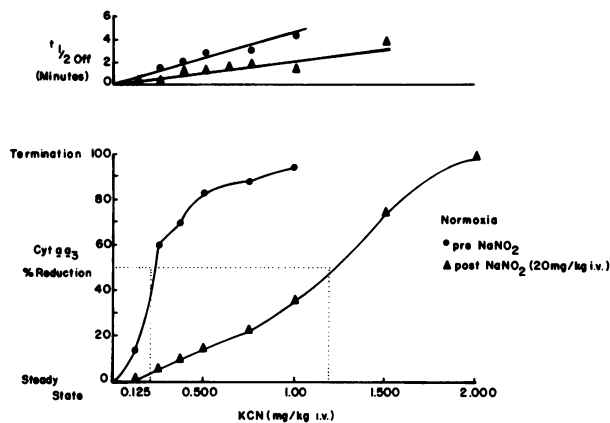


FIGURE 9 Effect of NaNO_2 treatment on cyanide-cytochrome dose-response curve. Lower plot was obtained as described in Fig. 4. Dotted lines show cyanide dose for 50% cytochrome oxidase reduction (K_D). Upper plot shows linear relationship between cyanide dose and time in min after KCN to 50% recovery of cytochrome redox transient ($t_{1/2 \text{ off}}$, Fig. 2).

oxidase-based explanation supporting either previous reports of decreased mortality in hyperoxic animals poisoned with cyanide (14-16) or oxygen antagonism of cyanide-induced electroencephalographic changes (43), our results do support careful experiments by Way et al. (17) demonstrating significant increases in LD_{50} for hyperoxic mice only when oxygen was used in combination with both sodium thiosulfate and sodium nitrite. Whether or not this effect of oxygen is somehow mediated through cytochrome *c* oxidase awaits further study with lethal doses of cyanide and appropriate antagonist combinations.

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