
10 Acute and Chronic Cyanide Toxicity*

Joseph L. Borowitz, Gary E. Isom, and Steven I. Baskin

CONTENTS

- I. [Introduction](#)
- II. [Cyanide Exposure](#)
- III. [Symptoms Produced by Cyanide](#)
- IV. [Chemical Reactivity of Cyanide](#)
- V. [Metabolism of Cyanide](#)
- VI. [Effects of Cyanide on Neural Tissue](#)
 - A. [Elevated Cell Calcium](#)
 - B. [Effects of Cyanide on Metabolism of Neurons](#)
 - C. [Oxidative Stress in Neuronal Cells and Cyanide](#)
 - D. [Hyperpolarization by Cyanide](#)
 - E. [Neuronal Activation by Cyanide](#)
- VII. [Effects of Cyanide on the Heart](#)
- VIII. [ADP-Ribosylation by Cyanide](#)
- IX. [Production of Cyanide in Neural Tissue](#)
- X. [Cyanide Antidotes](#)
- XI. [Summary](#)

[Acknowledgments](#)

[References](#)

I. INTRODUCTION

Since the previous report in 1992, important new findings have provided fresh insight into CN mechanisms of action in both neural and cardiac tissue, the primary targets of CN intoxication.¹ Most studies use CN to produce chemical hypoxia or to mimic conditions caused by stroke and myocardial infarction. Generally, actions of CN resemble those of ischemia and hypoxia, so information gained from CN studies is as important for the analysis of the chemical itself as for study of common pathological conditions.²⁻⁵

*The opinions or assertions contained in this paper are the private views of the authors and are not to be construed as official or as reflection of the views of the Army or Department of Defense.

Not only is CN acutely toxic in high (mg) doses, serious neurological problems are associated with chronic exposure at lower levels.⁶ This review also includes recent observations on this timely issue.

Finally, there is strong evidence that mammalian tissues actually produce CN,^{7,8} and it has been proposed that CN may serve as a neuromodulator.⁶ Much work needs to be accomplished to determine mechanisms by which neural and other tissues produce CN, and also the physiological and pathological significance of endogenous CN.

II. CYANIDE EXPOSURE

Recordings from antiquity show that Egyptians and Romans utilized CN-containing poisonous plant extracts as a chemical instrument for suicide or murder. Preparations of cherry laurel water containing cyanogenic glycosides distilled from the bark of the tree were utilized by Nero to dispose of individuals who displeased him.⁹

Napoleon III proposed the use of CN tipped bayonets during the Franco-Prussian war. Lord Playfair also sought to implement its use during the Crimean War. The brilliant German chemists such as Michaelis and Haber studied the kinetics of CN in the laboratory and their lessons were applied to the field. World War I experiences taught that CN could produce rapid death in the field, but the slow WWI munition delivery and manufacture of impure product did not allow for dependable dispersal of HCN as a munition.¹⁰ However, introduction of vincennite mixtures (shell No. 4) at the Somme and the method of rapid firing made it almost impossible to put masks on in time to protect against CN. Subsequent reports showed CN mixtures were far more effective than realized.¹⁰ More efficient delivery systems and improved methods of CN synthesis and storage may overcome the technical problems experienced in WWI. Magnum and Skipper reported from observations made during convict execution that man is incapacitated (onset of convulsions) by approximately 10 mg/L within 10 to 18 s.¹¹

At the beginning of WWII, CN was used by the Japanese forces on the Bataan Peninsula in the form of a hand grenade and in Manchuria and China for poisoning wells.¹¹ The Nazis used the poison at the beginning of WWII to kill entrenched Yugoslav partisans in caves (Adjimushkaiskye) and during WWII to exterminate over 2 million concentration camp inmates. In a chaotic 3-day period with the Russian forces approaching, Höss, the commandant of Auschwitz, increased the Zyklon B (hydrocyanic acid adsorbed onto a dispersible pharmaceutical base) concentration to accelerate the normal killing rate for inmates and to exterminate over 10,000 Russian soldiers. In the 1980s, several Middle Eastern sites were reported to be CN targets. The inhabitants of Hama, Syria were gassed as a part of a political solution, as were inhabitants of Halabja, Iraq, and possibly in Shahabad, Iran, during the Iran-Iraq war.¹²⁻¹⁴

Certain parameters of CN-induced lethality in man and other mammals have been examined for many years; however, because of its highly toxic and rapid-acting nature, much less is known about sublethal CN toxicity.¹⁵ It has been suggested that the central nervous system (CNS), in particular, is highly sensitive to the toxic effects of CN, and may be the primary target system.^{16,17} CNS changes due to

non-CN-induced (e.g., hypoxic) hypoxia resemble those induced by CN, although the latter also produce enzyme and neurotransmitter changes.¹⁵

It is interesting to note that CN is formed, exchanged with every breath we take, and exhaled at concentrations much less than what is considered toxic. The observations that man is constantly exchanging endogenous CN,⁷ and studies suggesting its function as a central modulator in rats in its gaseous state, similar to what has been seen for carbon monoxide,¹⁸ suggest that CN may be providing a biological role as a neuromodulator in addition to that of an exogenous synthetic poison.

Today, poisonings have taken place as a result of contact with or breathing of cleaning products for silver, which contain CN. Cyanide is also used in many industrial applications, such as electroplating, case hardening steel at ~900°C, mining, and agricultural fumigation. It is also used in products related to hydrogen cyanide [HCN] (hydrogen nitrile) by incorporation of other nitriles such as industrial solvents (acetonitrile [methylcyanide], for example) or nitrile polymers such as nylon. Thus, CN polymers have become useful items in everyday life. However, these same products can, like nylon, depolymerize in fire and release short-chain monomers or CN resulting in serious CNS toxicity or death. Cyanide is found in many forms and precursors that can be taken into the body. It was noted since antiquity that certain plants could produce a CNS respiratory gasp followed by anoxia, convulsions, occasionally culminating in death. (This breathing reflex appears to be one of the most sensitive responses to CN exposure.)

A wide variety of plant life incorporates nitrile-containing substances that are metabolically or chemically converted to CN, toxic to both animal and man.¹⁹ For example, a large number of plants in the *Rosecea* genus (e.g., cherry, peach, and bitter almond) are known to contain cyanogenic glycosides. Many of the behavioral and CNS effects of CN were originally observed after the ingestion of CN-containing plant products. Plants can contain cyanogenic lipids (for example, in *Sapandous drummondii*) or cyanogenic glycosides (for example, in cassava, sorghum, flax, white clover). Cassava (*Manihot esculenta*) is a common crop utilized as a foodstuff (manioc) in parts of Asia, South America, and Africa. If not properly processed, it can pose a serious cyanogenic hazard. The plant stores a cyanogenic glycoside, linamarin that is degraded by the enzyme linamarase to cyanohydrins and subsequently to hydrocyanic acid.

III. SYMPTOMS PRODUCED BY CYANIDE

High doses of CN are rapidly fatal, probably due to respiratory arrest. Severe CN poisoning disrupts neural mechanisms controlling consciousness and breathing, though the heart continues to beat (at a much slower rate which is probably incompatible with normal or life-sustaining function).⁹ Animals given high but sublethal doses of CN (e.g., mice with 5-mg/kg KCN s.c.; see Figure 10.1) become quiescent a few minutes after injection but may remain conscious and can respond to physical stimulation. After another few minutes, the animals appear to resume normal locomotor activity. These important symptoms reflect transient actions of CN on different neural

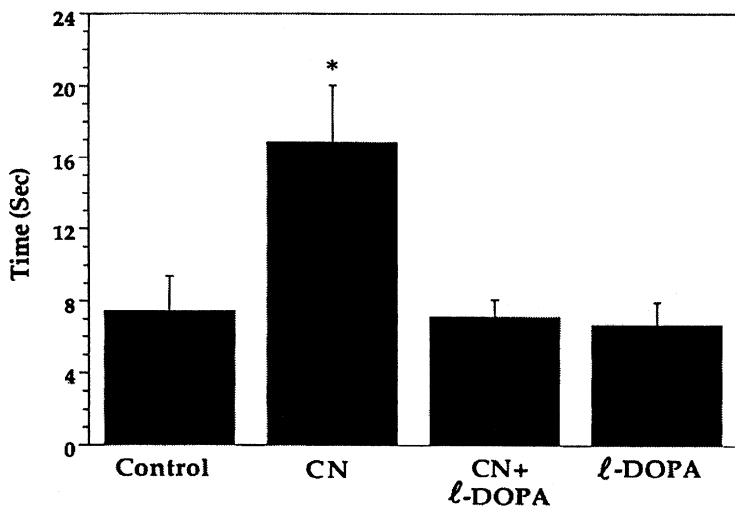


FIGURE 10.1 Production of catalepsy in mice by repeated CN treatment: Mice were treated with 6 mg/kg (s.c.) KCN twice a day for 7 days. Sixteen hours after the last dose, catalepsy was quantitated. In *l*-DOPA experiments, 100 mg/kg (i.p.) *l*-DOPA was administered 1 h prior to quantitation of the degree of catalepsy. Values are the means \pm SEM from four determinations and each determination consisted of three animals in each group. Asterisk indicates a significant difference from control at $p < 0.05$. (Reproduced with permission)

systems. Many recent studies discussed below reveal the complex nature of CN's effects on different neural pathways.

Cyanide stimulates chemoreceptor reflexes.²⁰ Denervation of the carotid sinus removes the respiratory stimulation and bradycardia. Using isolated cat carotid bodies or sinuses, dopamine produces a transient depression of the frequency of chemoreceptor discharges. The effect of dopamine can partially or totally antagonize the excitation of chemoreceptor discharges evoked by acetylcholine or CN.²¹ The effect of dopamine is long acting. Also, changes in the intracellular sodium and calcium concentration influence the excessive depolarization and sensory discharge of the cat carotid body and nerve produced by CN.²² Concentrations as low as 10–50 nM CN reduce cytochromes in the carotid body reflecting the extreme sensitivity of this tissue to CN. It is this site that appears to be responsible for the respiratory gasp. Fluorometry revealed reduction of NADH as well.^{23,24} Thus, the interaction with CN in the peripheral nervous system appears to be the most sensitive at the chemoreceptor site.

Studies are currently ongoing to identify the primary oxygen-sensing (and perhaps the CN-sensing) protein controlling transmitter release and electrical activity of the carotid sinus nerve. It is also suggested that this primary oxygen- and CN-sensing receptor is a hemeprotein that does not participate in mitochondrial energy production. A cytochrome b (558) was described for the NAD(P)H oxidase.²⁵ These results suggest that there may be a specific molecular site for the sensing of CN.

Non-lethal chronic exposure to CN can lead to neurological problems; some neurodegenerative diseases are associated with chronic CN treatment.⁶ Mice that are given potassium cyanide (6-mg/kg s.c. twice daily for 7 days) exhibit Parkinsonian symptoms of decreased motor activity and akinesia²⁶ (Figure 10.1). The reviews by Isom et al. and Baskin and Rockwood cover the relationship between CN ingestion and both the conditions of tropical ataxic neuropathy and the upper motor neuron disease “Konzo.”^{6,27} Furthermore, evidence of abnormal CN metabolism has been reported for tobacco amblyopia, Leber’s optic atrophy, and amyotrophic lateral sclerosis.²⁷ Symptoms seen in the epidemic of optic neuropathy in Cuba between 1991 and 1993, discussed by Isom et al. resemble those of tobacco amblyopia, and many cases have peripheral neuropathies as well, such as painful dyesthesias and decreased ankle reflexes.²⁷ Vitamin B₁₂ is used to treat tobacco amblyopia according to the concept that B₁₂ deficiencies increase susceptibility to CN in tobacco smoke. The optic neuropathy in Cuba affected 50,000 people and was also effectively treated with B₁₂.²⁷ Sudan suggests that vitamin deficiencies, exposure to methanol, and CN contributed to the Cuban epidemic, and that defective mitochondrial function impairs ATP production even to the extent of interfering with axonal transport of mitochondria to nerve endings.²⁸

Epidemiological studies reveal a high incidence of Parkinsonism occurring in rural areas.^{29,30} More recently, Hobson et al. have noted a direct relationship between use of calcium CN dust and Parkinsonism in beekeepers.³¹ Cyanide as an environmental factor appears to be important in some neurological disorders.²⁷

IV. CHEMICAL REACTIVITY OF CYANIDE

Cyanide (hydrocyanic acid, HCN) is a small molecule with good lipid and water solubility. Physically, it can exist as a gas or liquid; it is miscible with water and slightly soluble in ether. Like nitric oxide and carbon monoxide, it easily penetrates biological membranes and acts intracellularly.⁹ At physiological pH, over 98% of the molecule is in the form of HCN and only a small fraction occurs as CN. The major biological effects are most likely due to the undissociated molecule. Cyanide strongly interacts with iron in protein molecules, inhibiting enzymes including carbonic anhydrase and succinic dehydrogenase.³² Formation of cyanhemoglobin by interaction of CN with ferric iron abolishes the ability of hemoglobin to carry oxygen. Interaction of CN with the ferric iron in mitochondrial cytochrome oxidase blocks cellular respiration; this has long been considered an important toxic action of CN.³³ Sun et al. also suggest that interaction of CN with disulfide groups on the NMDA receptor regulatory sites enhances receptor function.³⁴ Arden et al. reported that CN acts on the NMDA receptor as a reducing agent to potentiate NMDA-induced electrical activity in rat cortical neurons, though an oxidizing agent reverses this action.³⁵ Cyanide is thought to potentiate glutamate neurotoxicity by this mechanism; however, how glutamate-CN interactions relate to CN’s *in vivo* toxicity is not completely established. Thus, the primary chemical interactions of CN are thought to involve ferric iron and disulfide bonds.

V. METABOLISM OF CYANIDE

In contrast to other chemical warfare agents, CN appears biologically in blood, urine, and expired breath.⁷ It is actually generated in small amounts in neuronal tissue, and researchers have proposed that CN functions as a neuromodulator similar to nitric oxide.²⁷ Cyanide contrasts with nitric oxide in that it is chemically more stable and is not immediately broken down. Enzymes exist that regulate CN concentrations and two sulfurtransferases, rhodanese and 3-mercaptopyruvate sulfurtransferase, as well as thiosulfate reductase, convert CN to thiocyanate, which is about seven times less toxic.³⁶ These enzymes account for 60–70% of the metabolism of non-toxic concentrations of CN and may act in concert since they have different tissue distributions. Rhodanese occurs in highest concentration in the liver with high levels also in kidneys, adrenals, and thyroid, whereas mercaptopyruvate sulfurtransferase has a broad tissue distribution with high levels in the liver, kidneys, and heart. Being lipid soluble and relatively stable, CN probably accumulates in lipid depots throughout the body, and is also bound to an albumin-binding site.³⁷ Mobilization from lipid and

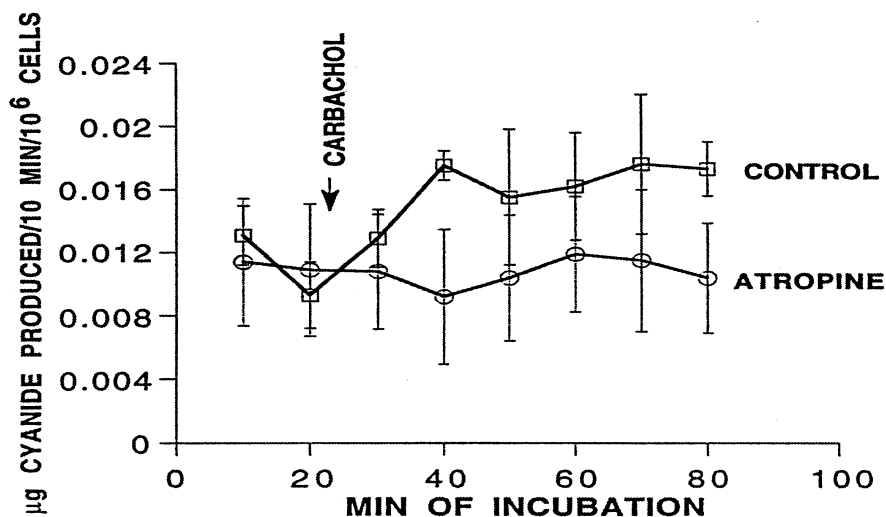


FIGURE 10.2 Blockade of carbachol-induced CN production in undifferentiated rat pheochromocytoma cells by atropine: Atropine 500 µM was added at the beginning of the experiment and carbachol (100 µM) was added after 20 min to both atropine and control samples. Air 95%, CO₂ 5% was passed over the cells and bubbled through 0.1 M NaOH to trap the CN. Aliquots of the NaOH were taken to measure CN colorimetrically (Lambert, J., Ramasamy, J., and Pakstelis, J., *Anal. Chem.* 47, 916, 1975. With permission). Note atropine completely blocked the response to carbachol but basal CN production was not affected by atropine. Apparently the cells generate CN from an atropine insensitive source which includes release from lipid depots and from proteins.

release from protein binding is suggested to account for some of the generation of CN detected in neural tissue (Figure 10.2).

A minor (approximately 20% under non-toxic conditions) but toxicological metabolic pathway (that may increase during CN poisoning) for CN involves the disulfide cystine. 2-ICA, or its tautomer 2-aminothiazolidine-4-carboxylic acid (2-ACA), is a detoxification product of CN that is formed by what is thought to be a non-enzymatic reaction of CN with cystine.³⁸ Cyanide reacts with cystine producing β -thiocyanoalanine, which spontaneously undergoes ring closure to form 2-ICA and its tautomer 2-ACA (Figure 10.3). These tautomers are in rapid chemical equilibrium and exist in equal concentration in solution. The formation of 2-ICA may increase with increased exposure to CN. One mechanism for this increase may be the decreased pH in the cells that favor the formation of 2-ICA compared with the maximal activity of the sulfurtransferases at a much higher pH.

Only limited research has been conducted to study *in vivo* formation of 2-ICA following systemic administration of CN.^{39,40} Depending on species, sensitivity of the assay and CN exposure conditions, the reported percentage of CN converted to 2-ICA ranges from 5–15% of delivered CN dose.^{40,41} 2-ICA does not appear to be metabolized, but is excreted slowly in the urine and saliva.^{38,41} We have studied its biological activity (i.e., memory loss, convulsions, loss of consciousness) and concluded 2-ICA contributes to the CNS actions of CN.^{42–44} The toxicokinetics of 2-ICA formation and its elimination (half-life) have not been determined. However, in a preliminary study of 2-ICA as a CN biomarker, Lundquist et al. showed 2-ICA was detectable in the urine by HPLC assay up to 4 weeks after administration of acetonitrile, a cyanogenic compound that is metabolized to CN.³⁹ In smokers or human subjects ingesting cyanogenic compounds, 2-ICA was detected in urine. In isolated rat hepatocytes, Huang et al. prevented cell death by 400 μ M CN using 1 mM cystine.⁴⁵ They found thiocyanate levels were also increased under these conditions, so the cystine may provide sulfur for thiocyanate formation as well as for 2-ICA production.

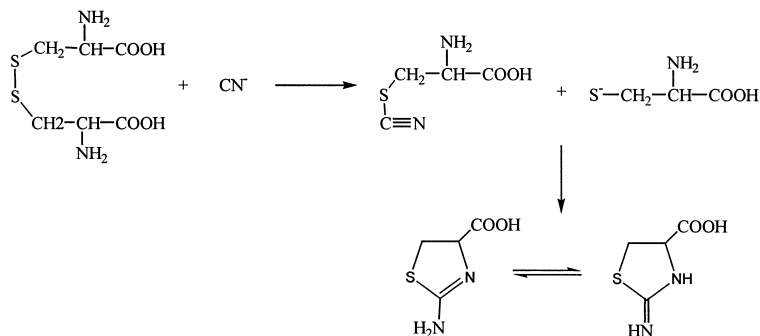


FIGURE 10.3 Conversion of cyanide to 2-aminothiazolidine-4-carboxylic acid or 2-iminothiazolidine-4-carboxylic acid.

VI. EFFECTS OF CYANIDE ON NEURAL TISSUE

A. ELEVATED CELL CALCIUM

Since the previous review, several significant papers further implicating elevated cellular calcium in CN-induced neurotoxicity have appeared.¹ Ferger and Kriegstein exposed chick telencephalic neurons to 1 mM NaCN for up to 2 h.⁴⁶ Increases in $[Ca]_i$ were measured with Fura-2, and viability was estimated by trypan blue exclusion. Elevation of $[Ca]_i$ paralleled neuronal damage.⁴⁶ On the other hand, insertion into PC12 cells of a herpes simplex vector expressing cDNA for calbindin did not prevent the rise in calcium or cell survival after exposure to 1–5 mM sodium CN (18 h) even though these calbindin-containing cells were protected against the effects of glutamate.⁴⁷ Compared to the neurotoxicity of cyanide, glutamate-induced neurotoxicity may be more intimately related to increases in cell calcium.

Two other reports suggest that calcium must be taken up into mitochondria to mediate toxicity. Thus, glutamate is not toxic to cultured rat forebrain neurons when uptake of calcium into mitochondria is inhibited.⁴⁸ An associated increase in cytosolic calcium occurs however despite the lowered toxicity. The authors suggest calcium is toxic only when it enters mitochondria and that high levels of cytosolic calcium do not appear to be toxic.⁴⁸ In support, Sengpiel et al. report that 1 mM sodium CN (admittedly a high concentration) prevented mitochondrial calcium uptake and reduced both neurotoxicity of NMDA in cultured rat hippocampal neurons and the associated NMDA-induced superoxide production.⁴⁹

B. EFFECTS OF CYANIDE ON METABOLISM OF NEURONS

CA1 hippocampal neurons are preferentially susceptible to hypoxia and ischemia. In CA1, CA3, and dentate gyrus neurons dissected from fresh rat hippocampal slices, CN specifically enhanced release of acid metabolic products from CA1 cells but had little effect on the other cells.⁵⁰ By contrast, kainate, which has CA3-specific effects, increased acid metabolite release only in CA3 neurons.⁵⁰ Actions of CN appear to be metabolic in nature and not all neuronal cell types are equally affected.

Zu and Krnjevic studied CN in hippocampal slices.⁵¹ They found 300 μ M CN did not block electrical responses to field stimulation as long as glucose levels were elevated to 10 mM, but in 4 mM glucose (physiological level), CN caused a characteristic hypoxic injury potential followed by a blockade of the response to electric fields. Intracellular recordings reveal a continued hyperpolarization in response to CN in 10 mM glucose, but in 4 mM glucose only a brief hyperpolarization occurred, followed by a major and usually irreversible depolarization. The authors suggested a reduced supply of ATP impairs restoration of membrane potential and causes the irreversible depolarization.

C. OXIDATIVE STRESS IN NEURONAL CELLS AND CYANIDE

Isom et al. reviewed mechanisms of apoptotic or necrotic neural damage caused by CN.²⁷ Cyanide-induced calcium entry by way of voltage-sensitive calcium channels

or NMDA receptors has three main actions. First, activation of lipases in the cell membrane increases arachidonic acid release, which leads to increases in reactive oxygen species. Calcium then activates nitric oxide synthase to increase nitric oxide levels. Finally, calcium activates proteases, lipases, and endonucleases that can damage structural and functional elements in neuronal cells. Reactive oxygen species and nitric oxide also can form peroxynitrite by reacting with superoxide. Peroxynitrite is a powerful oxidant which has many and varied effects in neurons and other cell types, including depletion of cell thiol groups, lipid peroxidation, mobilization of cell calcium, impaired mitochondrial function correlated with muscular contractile failure in rat diaphragms, and modification of synaptic proteins.^{52–57} Uric acid is a peroxynitrite scavenger and protects cells against this powerful oxidant. In granule cells of the cerebellum, uric acid protects against CN-induced apoptotic death indicating that peroxynitrite is an important mediator of cell damage by CN.^{58,59}

D. HYPERPOLARIZATION BY CYANIDE

Cyanide causes either a hyperpolarization or a depolarization when tested on neuronal tissue depending on conditions and type of neurons involved. Hippocampal CA1 neurons usually hyperpolarize on exposure to hypoxia, but hypoglossal neurons depolarize under the same conditions.^{60–62} The hyperpolarization may be a protective mechanism to prevent activation of the cell in a time of stress.⁶³ Usually the potassium channels involved are ATP regulated (K_{ATP}), but this also varies with the cell type. In undifferentiated rat pheochromocytoma cells, hyperpolarization occurs due to opening of K_{Ca} channels subsequent to an increase in $[Ca^{2+}]_i$.⁶⁴ In dissociated rat locus coeruleus neurons, the hyperpolarization caused by sodium CN involves both IK_{ATP} and IK_{Ca} .⁶⁵ Studying neurons in rat locus coeruleus slices, Yang et al. found 61% of the neurons hyperpolarized when treated with 2 mM CN (albeit, a large amount of CN) but 39% of the neurons depolarized.⁶⁶ Thus, in neurons responsible for sending noradrenergic impulses throughout the CNS from the same tissue, the response to histotoxic anoxia is variable. Yang et al. suggest that distribution of K_{ATP} channels among neurons of the locus coeruleus is variable since the K_{ATP} channel-opener diazoxide could mimic the hyperpolarizing effect of CN in 61% of the neurons, but not in the 39% depolarized by CN.⁶⁶

In a test of the concept that hyperpolarization protects neurons from toxic damage, a potassium channel opener, bimakalim, was employed and was found to protect embryonic chick telencephalic neurons from 1 mM CN-induced injury. The protective effect of bimakalim was canceled by the K_{ATP} blocker tolbutamide.⁶³ Apparently the extent of the hyperpolarization caused by CN is not sufficient to give optimal protection and a further increase in neuronal polarity provides even more damage control.

Also in hippocampal slices using high glucose (11 mM), Zhu and Krenjevic report that the inhibitory effect of 100 μ M KCN was blocked by adenosine antagonists, potentiated by the adenosine uptake blocker dipyrindamole but was not affected by glyburide, a K_{ATP} channel blocker.⁶⁷ They suggest that adenosine release may be a major cause of the early depression of CNS function caused by CN. Adenosine is known to be released from nerve cells by CN, and to cause hyperpolarization by a

G protein effect on potassium channels.⁶⁸⁻⁷¹ Adenosine release by CN must be considered a factor in CN-induced neural injury.

E. NEURONAL ACTIVATION BY CYANIDE

Exposure of freshly excised rat CA1 hippocampal neurons to 5 mM CN increased the $I_{Na,P}$ sodium current but had no significant effect on the amplitude of the more transient current $I_{Na,T}$.⁷² Bubbling 100% N_2 into the medium similarly increased $I_{Na,P}$ indicating that CN and hypoxia have similar mechanisms. Persistent increase in sodium current probably explains the increased $[Na^+]_i$ seen in cortical neurons during hypoxia.⁷³ The $I_{Na,P}$ caused by CN was blocked by tetrodotoxin or lidocaine. Persistent flow of sodium through sodium channels may activate voltage-sensitive calcium channels or activate the Na^+/Ca^{++} exchanger, to increase $[Ca^{2+}]_i$. Thus an increase in $I_{Na,P}$ may be the initial event caused by hypoxia leading to cell death. In fact sodium channel blockers can block the $[Ca^{2+}]_i$ increase and prevent cell damage during hypoxia.⁷⁴ In support, procaine protects mice against the lethal effects of CN.⁷⁵ Combination of procaine with sodium nitrite and sodium thiosulfate enhanced the effectiveness of the nitrite/thiosulfate treatment. Furthermore, the CN-induced increase in whole mouse-brain calcium from 28 to 48 mg/g dry weight was also blocked by procaine pretreatment.⁷⁵ Abnormal sodium channel function may be a primary event in CN-induced neuronal damage.

VII. EFFECTS OF CYANIDE ON THE HEART

The previous review mentioned CN-induced changes in myocardial, calcium, and H^+ as factors in myocardial depression caused by this agent. Marked CN-induced increases in circulating catecholamine stimulate the heart,⁷⁵ but, at the same time, energy metabolism is impaired and heart failure results.⁷⁶

How CN decreases cardiac contractility is important and has been studied by several groups. Hydrogen ion accumulation contributes to the lack of effectiveness of $[Ca^{++}]_i$ in activating the contractile process.⁷⁷ Blockade of oxidative metabolism by CN increases glycolysis and therefore increases lactic acid production. Because ATP is continuously broken down, inorganic phosphate (P_i) accumulates since less is being used to make ATP. Increases from 4 to 10.5 mM P_i have been measured in CN-treated perfused ferret hearts.⁷⁸ Essentially, this provides heart cells with added phosphate buffer to minimize pH changes. Changes of only 0.2 unit were noted in ferret hearts perfused with 1 mM CN, or 0.08 units in rat hearts perfused with 1 mM KCN.^{79,80} Even though hydrogen ion accumulation is not large, it explains some of the decreased myocardial contractility caused by CN. Hydrogen ion is a strong competitor with calcium for binding sites in tissues.⁸¹ Effects of pH may be more noticeable in intact hearts compared to isolated myocytes because of differences in the rate at which lactic acid can leave the tissue.⁸⁰

Cytosolic calcium overload is generally associated with cell injury and energy deprivation increases intracellular calcium.^{82,83} Kondo et al. measured 2 mM

CN-induced increases in systolic (104% above control) and diastolic (37%) calcium in paced rat myocytes.⁷⁷ Despite the increase in calcium, contractile function decreased to 58% of control. Doubling extracellular calcium, restored contractility to 123% of control and increased systolic (225% above control) and diastolic (73%) $[Ca^{++}]_i$. However no increase in cell damage was noted over a period of 40 min (25% of the cells went into contracture when exposed to normal $[Ca^{++}]_o$ and 2 mM CN and incidence of damage was the same in high calcium). These observations have important implications. First, the decrease in contractility was related to the relative ineffectiveness of $[Ca]_i$ to activate the contractile machinery, partly due to elevated hydrogen ion. When calcium was further increased, contraction was fully restored. Second, reduced energy availability does not appear to be a problem at least when an abundance of glucose (19.5 mM) was provided. The hearts functioned well when $[Ca^{++}]_o$ was increased despite the continued presence of CN, and remarkably no greater increase in contracture or increase in cell destruction occurred.

Kupriyanov et al. perfused rat hearts with 1 mM KCN and showed decreased heart rate and perfusion pressure associated with an increase in osmolarity.⁷⁹ Increases in P_i occur when ATP is broken down, 3 P_i are formed, and the nucleoside leaves the cell; phosphocreatine is also broken down to further increase P_i levels. Breakdown of glycogen to lactate also contributes to the overall increase in osmolarity estimated to be about 26 mM. Some increase in intracellular water (~10%) would be expected in CN-treated heart and this cellular edema may affect function.

Kupriyanov et al. also noted an increase in $[Na^+]_i$ and a decrease in $[K^+]_i$ in rat hearts perfused with 1 mM KCN.⁷⁹ Decreased Na^+K^+ ATPase activity due to decreased ATP levels could explain this change. However, it is reported that even a 20-fold decrease in cytoplasmic ATP/ADP does not decrease Na^+K^+ ATPase activity in perfused rat heart,⁸⁴ so the 5-fold decrease observed by Kupriyamov et al. cannot explain the increased $[Na^+]_i$.⁷⁹ These authors suggest that Na^+K^+ ATPase is inhibited by the increased P_i , which can form a ternary abortive complex with the enzyme and ADP.

Cyanide activates K_{ATP} channels in the brain and also in the heart.^{63,79} The K_{ATP} channel inhibitor glibenclamide blocked the effect of KCN in the Langendorf perfused rat heart.⁷⁹ However part of the effect of glidenclamide and that of KCN on cell potassium is due to inhibition of Na^+K^+ ATPase. An increase in K^+ loss through the K^+ /lactate co-transporter by KCN was also demonstrated by use of a blocker of this transport system, α -cyano-4-hydroxycinnamic acid. Thus the effect of KCN on K^+ efflux in the heart involves three factors: activation of the K_{ATP} channel, blockage of Na^+K^+ ATPase, and activation of the K^+ lactate cotransporter.

The diaphragm is similar to the heart in that it also responds rhythmically to stimulation. After a brief potentiation of muscle twitch, CN (0.1–1 mM) causes a slow progressive depression of contractility of the rat diaphragm.⁸⁵ Potentiation is due to an increase in pH from replenishment of ATP by phosphocreatine (creatine kinase mediated transphosphorylation of ADP to ATP). Inhibition of muscle twitch is due to lactate accumulation as well as increased P_i and increased $[Mg^{2+}]_i$ from breakdown of magnesium phosphocreatine.⁸⁵ No decreases in ATP or action potential generation were caused by CN treatment in rat diaphragms.⁸⁵ Because skeletal muscle,

including the diaphragm, is less active than heart muscle, it is also less sensitive to metabolic inhibition by CN.

VIII. ADP-RIBOSYLATION BY CYANIDE

Proteins may be modified posttranslationally by transfer of the ADP-ribose moiety of nicotinamide adenine dinucleotide to an amino acid. Five mammalian ADP-ribosyl transferases (ART-I-ART-5) have been cloned and expression is limited to certain tissues including heart and brain.⁸⁶ These transferases are regulated by ADP-ribosylation factors (ARF) which are small monomeric G proteins activated by combination with GTP.⁸⁷ The system is stimulated by reactive oxygen species and may protect cells from oxidative damage or may influence the type of death a cell undergoes.^{88–90}

It was reported in 1988 that CN increases ADP ribosylation of mitochondrial proteins.⁹¹ Surprisingly, this interesting effect has not been studied further. Some of the observed actions of CN such as enhanced neurotransmitter release and alignment of chromaffin granules along the plasma membrane may be explained by ADP-ribosylation of certain proteins, since protein ribosylation can affect exocytosis from chromaffin cells and membrane recycling in the Golgi apparatus.^{92,93}

A similar process involves poly (ADP-ribose) polymerase (PARP), which catalyses attachment of multiple ribose units from NAD to nuclear proteins. Genetic disruption of PARP protects against ischemic insults *in vitro* and limits infarct volume after reversible middle cerebral artery occlusion in mice.⁹⁴ Apparently excessive PARP activation in ischemia depletes NAD and ATP (which regenerates NAD) and causes cell death by energy depletion.⁹⁴ It would seem that PARP is certainly involved in the action of CN on neural tissue but no such work has been reported.

IX. PRODUCTION OF CYANIDE IN NEURAL TISSUE

Isom et al. mentioned endogenous generation of CN and the possibility that CN may function as a neuromodulator in a manner similar to nitric oxide.²⁷ Brain CN levels are increased by hydromorphone and the effect is blocked by naloxone.²⁷ Undifferentiated rat pheochromocytoma cells also show increased CN production in response to hydromorphone or morphine.²⁷ Since PC12 cells have mainly kappa opiate receptors and no mu receptors, hydromorphone probably acts through kappa receptors to increase CN release.⁹⁵

If CN is indeed a neuromodulator, it contrasts with nitric oxide. Except for conversion to thiocyanate by sulfurtransferase enzymes, CN is relatively stable in biological systems and exists to the extent of about 3 μM in human blood.^{96,97} Those who smoke have elevated blood CN levels. Nitric oxide, on the other hand, spontaneously breaks down in biological fluids, having a half-life of a few seconds.⁹⁸ Thus CN can accumulate in biological materials, collecting in lipoid depots since it is lipid soluble. Cyanide also forms complexes with albumin through addition to disulfide bonds, and one study proposed this interaction to be a mechanism to remove CN from blood.⁹⁹

Cyanide may interact with proteins in other ways by forming hydrogen bonds or salt bridges with appropriate sites on protein molecules. Cyanide in lipid membranes or bound to protein may be in equilibrium with free CN in biological fluids.

Whether disturbances in CN generation or metabolism can cause disease is controversial, although CN imbalance is implicated in Leber's optic atrophy and amyotrophic lateral sclerosis.²⁷ Important work remains to be done to determine the role of endogenous CN in physiological systems and in disease states.

X. CYANIDE ANTIDOTES

Cyanide is a powerful intracellular poison that acts rapidly due to its good lipid and water solubility, and can quickly cause profound hypoxia in vital organs resulting in death. Prompt diagnosis and timely, effective use of antidotes is critical for the severely poisoned patient.

In the United States, the only Food and Drug Administration-approved antidote is the Cyanide Kit currently manufactured by Taylor Pharmaceutical Co. It actually contains three antidotes: amyl nitrite, sodium nitrite, and sodium thiosulfate. The nitrites form methemoglobin, which is an avid scavenger of CN. They also may give rise to nitric oxide, which is an effective CN antidote independent of methemoglobin formation.¹⁰⁰ Amyl nitrite is a volatile liquid; the glass vial containing the drug is crushed in gauze to allow inhalation by the comatose patient. Sodium nitrite is then given slowly (i.v.) for more extensive methemoglobin generation. Thiosulfate is a sulfur donor aiding the sulfur transferase enzymes, rhodanese and 3-mercaptopyruvate sulfurtransferase, which convert CN to thiosulfate, a much less toxic substance.

Cobalt diedetate (Kelocyanor) is well known in Europe and popular as a CN antidote. It is not available in the United States. Adverse effects of the antidote are seizures, angioedema, cardiovascular instability, and gastrointestinal problems. However, cobalt is a rapid-acting antidote and effective even in the severely poisoned patient.

Thiosulfate enhances the antidotal effect of many substances other than the nitrites. As mentioned in the 1992 review, α -ketoglutarate is a potential antidote with few side reactions and good effectiveness against the toxic effects of CN.¹ Its activity is markedly enhanced when given in combination with thiosulfate.¹⁰¹

XI. SUMMARY

In conclusion, low-level acute exposure to CN has been characterized by a respiratory gasp, which is believed to be caused by stimulation of chemoreceptors in the aortic arch. The chronic consequences of this type of acute exposure to CN are largely unknown. Since there are normal cellular mechanisms that maintain the balance between CN and sulfur, the equilibrium of the systems is thought to be well controlled.

Low-level chronic exposure to CN has not been fully characterized. It is believed that enzymes modulate and regulate CN and sulfur turnover at the cellular level to try

to maintain homeostasis. Studies of overload of the regulatory balance systems need to be systematically undertaken to determine which enzymes compensate as feedback compensation.

CN does not uniformly affect all brain cells. CA1 neurons in the hippocampus are more susceptible than CA3 cells to metabolic inhibition by CN. Certain neuronal type cells, e.g., those in the carotid body, are highly sensitive to the actions of CN. Thus, CN's actions on the neural systems are complex and depend on the type of neuron involved. Most likely, some nerve pathways are activated while others are inhibited or unaffected when an individual is exposed to CN.

ACKNOWLEDGMENTS

The authors thank Mr. Pinal C. Patel and the library staffs at the U.S. Army Medical Research Institute of Chemical Defense and Purdue University.

REFERENCES

1. Borowitz, J.L., Kanthasamy, A.G., and Isom, G.E., *Chemical Warfare Agents*, Somani, S., Ed., Academic Press, New York, 1992, 209.
2. Ballanyi, K. and Kulik, A., Intracellular Ca^{2+} during metabolic activation of K_{ATP} channels in spontaneously active dorsal vagal neurons in medullary slices, *Eur. J. Neurosci.*, 10, 2574, 1998.
3. Hammerstrom, A.K. and Gage, P.W., Inhibition of oxidative metabolism increases persistent sodium current in rat CA1 hippocampal neurons, *J. Physiol.*, 510, 935, 1998.
4. Yang, J.J., Chou, Y.C., Lin, M.T., and Chiu, T.H., Hypoxia-induced differential electrophysiological changes in rat locus coeruleus neurons, *Life Sci.*, 61, 1763, 1997.
5. Inoue, M., Fujishiro, N., and Imanaga, I., Hypoxia and CN induce depolarization and catecholamine release in dispersed guinea-pig chromaffin cells, *J. Physiol.*, 507, 807, 1998.
6. Isom, G.E., Gunasekar, P.G., and Borowitz, J.L., *Chemicals and Neurodegenerative Disease*, S. Bondy, Ed., Prominent Press, Scottsdale, AZ, 1999, 101.
7. Lundquist, P., Rosling, H., and Sorbo, B., The origin of hydrogen CN in breath, *Arch. Toxicol.*, 61, 270, 1988.
8. Borowitz, J.L., Gunasekar, P.G., and Isom, G.E., Hydrogen cyanide generation by μ opiate receptor activation: Possible neuromodulatory role of endogenous CN, *Brain Res.*, 768, 294, 1997.
9. Sollmann, T., *A Manual of Pharmacology and Its Applications to Therapeutic and Toxicology*, 7th ed., W. B. Saunders Co., Philadelphia, PA, 1948.
10. Macy, R., *Hydrocyanic Acid: Its Military History and a Summary of Its Properties*, Edgewood Arsenal, MD, Chemical Warfare Service, War Department. (DTIC No. AD-B957 032), 1937.
11. Magnum, G.H. and Skipper, H.E., Hydrocyanic acid: The toxicity and speed of action on man, *Edgewood Arsenal Memorandum Report* (Project A 3.5-1), Aberdeen Proving Ground, MD, (T.D.M.R. 471), 1942.
12. Lang, J.S., Mullin, D., Fenyvesi, C., Rosenberg, R., and Barnes, J., Is the "protector of lions" losing his touch? *U.S. News World Rep.*, 10, 29, November 1986.
13. Anonymous, Medical experts use of chemical weapons in Iran-Iraq war, *UN Chronicle*, 22, 24, 1985.

14. Heylin, M., Ed., U.S. decries apparent chemical arms attack, *Chem. Eng. News*, 66, 23, 1988.
15. D'Mello, G.D., Neuropathological and behavioral sequelae of acute CN toxicosis in animal species, in *Clinical and Experimental Toxicology of Cyanides*, Ballantyne, B. and Marrs, T.C., eds., Bristol, UK, Wright, 1985, 156.
16. Way, J.L., Mechanism of CN intoxication and its antagonism, *Fund. Appl. Toxicol.*, 3, 339, 1983.
17. Way, J.L., Sylvester, D., Morgan, R.L., Isom, G.E., Burrows, G.E., Tamulinas, C.B., and Way, J.L., Recent perspectives on the toxicodynamic basis of CN antagonism, *Fund. Appl. Toxicol.*, 4, S231, 1984.
18. Borowitz, J., Gunasekar, P., and Isom, G., Hydrogen cyanide generation by mu opiate receptor activation: Possible neuromodulatory role of endogenous cyanide, *Brain Res.*, 768, 294, 1997.
19. Evered, M.D., Robinson, M.M., and Rose, P.A., Effect of arterial pressure on drinking and urinary responses to angiotensin II, *Am. J. Physiol.*, 254, R69, 1988.
20. Heymans, C., Bouckaert, J.J., and Dautrebande, L., Sinus carotidien et reflexes respiratoires. III: Sensibilité des sinus carotidiens aux substances chimiques. Action stimulante respiratoire réflexe du sulfure de sodium, du cyanure de potassium, de la nicotine et de la lobeline, *Arch. Int. Pharmacodyn. Théra.*, 40, 54, 1931.
21. Zapata, P., Effects of dopamine on carotid chemo- and baroreceptors in vitro, *J. Physiol.*, 244, 235, 1975.
22. Eyzaguirre, C. and Nishi, K., Effects of different ions on resting polarization and on the mass receptor potential of carotid body chemosensors, *J. Neurobiol.*, 7, 417, 1976.
23. Acker, H., Eyzaguirre, C., and Goldman, W.F., Redox changes in the mouse carotid body during hypoxia, *Brain Res.*, 330, 158, 1985.
24. Lahiri, S., Ehleben, W., and Acker, H., Chemoreceptor discharges and cytochrome redox changes of the rat carotid body: Role of heme ligands, *Proceedings of the National Academy of Science USA*, 96, 9427, 1999.
25. Acker, H., Mechanisms and meaning of cellular oxygen sensing in the organism, *Resp. Physiol.*, 95, 1, 1994.
26. Kanthasamy, A.G., Borowitz, J.L., Pavlakovic, G., and Isom, G.E., Dopaminergic neurotoxicity of CN: Neurochemical, histological and behavioral characterization, *Toxicol. Appl. Pharmacol.*, 126, 156, 1994.
27. Baskin, S.I. and Rockwood, G.A., *Neurotoxicological and Behavioral Effects of Cyanide and Its Potential Therapies*, in press.
28. Sudan, A., Acquired mitochondrial impairment as a cause of optic nerve disease. Transaction Am., *Ophth. Soc.*, 96, 881, 1998.
29. Lanston, J.W., Epidemiology versus genetics in Parkinson's disease, *Ann. Neurol.*, 44 (suppl), S45, 1998.
30. Rajput, A.H., Uitti, R.J., and Rajput, A., Neurological disorders based on provincial health case records, *Neuroepidemiology* 7, 145, 1998.
31. Hobson, D.E., Del Bigio, M.R., and Stoss, B.J., Beekeeper Parkinsonism: A consequence of chronic intermittent CN poisoning, submitted for publication.
32. Ballantyne, B., *Clinical and Experimental Toxicology of Cyanides*, Ballantyne, B. and Marrs, T.C., eds., IOP Publishers, Bristol, England, 1987, 41.
33. Way, J.L., Cyanide intoxication and its mechanism of antagonism, *Ann. Rev. Pharmacol. Toxicol.*, 24, 51, 1984.
34. Sun, P.W., Rane, S.G., Gunasekar, P.G., Borowitz, J.L., and Isom, G.E., Cyanide interaction with redox modulatory sites enhances NMDA receptor responses, *J. Biochem. Molec. Toxicol.*, 13, 253, 1999.

35. Arden, S.R., Sinov, J.D., Potthoff, W.K., and Aizenman, E., Subunit specific interactions of CN with the N-methyl-D-aspartate receptor, *J. Biol. Chem.*, 293, 21505, 1998.
36. Isom, G.E. and Baskin, S.I., *Comprehensive Toxicology*, Vol. 3, Sipes, G., McQueen, C.A., and Gandolfi, A.J., Eds., Pergamon Press, Cambridge, UK, 1997, 477.
37. Lieske, C.N., Clark, C.R., Zoeffel, L.D., von Tersch, R.L., Lowe, J.R., Smith, C.D., Broomfield, C.A., Baskin, S.I., and Maxwell, D.M., Temperature effects in cyanolysis using elemental sulfur, *J. Appl. Toxicol.*, 16(2), 171, 1996.
38. Wood, J.L. and Cooley, S.L., Detoxification of cyanide by cystine, *J. Biol. Chem.*, 218, 449, 1956.
39. Lundquist, P., Kagedal, B., Nilsson, L., and Rosling, H., Analysis of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine by high-performance liquid chromatography, *Anal. Biochem.*, 228, 27, 1995.
40. Swenne, I., Eriksson, U.J., Christoffersson, R., Kagedal, B., Lundquist, P., Nilsson, L., Tylleskar, T., and Rosling, H., Cyanide detoxification in rats exposed to acetonitrile and fed a low protein diet, *Fund. Appl. Toxicol.*, 32, 66, 1996.
41. Ruzo, L.O., Unai, T., and Casida, I.E., Decamethrin metabolism in rats, *J. Agric. Food Chem.*, 26, 918, 1978.
42. Weuffen, W., Jess, G., Julich, W.D., and Bernhardt, D., Untersuchungen zur Beziehung zwischen der 2-Iminothiazolidin-4-carbon säure und dem thiocyanatstoffwechsel des Meerschweinchens, *Die Pharmazie*, 35, 221, 1980.
43. Bitner, R.S., Kanthasamy, A., Isom, G.E., and Yim, G.K.W., Seizures and selective CA-1 hippocampal lesions induced by an excitotoxic cyanide metabolite, 2-iminothiazolidine-4-carboxylic acid, *Neurotoxicology*, 16, 115, 1995.
44. Bitner, R.S., Yim, G.K.W., and Isom, G.E., 2-Iminothiazolidine-4-carboxylic acid produces hippocampal CA-1 lesions independent of seizure excitation and glutamate receptor activation, *Neurotoxicology*, 18, 3215, 1997.
45. Huang, J., Niknahad, H., Kahn, S., and O'Brien, P.J., Hepatocyte-catalysed detoxification of CN by L- and D-cysteine, *Biochem. Pharmacol.*, 55, 1983, 1998.
46. Ferger, D. and Kriegelstein, J., Determination of intracellular Ca^{2+} concentration can be a useful tool to predict neuronal damage and neuroprotection properties of drugs, *Brain Res.*, 932, 87, 1996.
47. Meier, T.J., Ho, D.Y., Parks, T.S., and Sapolsky, R.M., Gene transfer of calbindin A28K < DNA via herpes simplex virus amplicon vector decreases cytoplasmic calcium ion response and enhances neuronal survival following glutamatergic challenge but not following CN, *J. Neurochem.*, 71, 1013, 1998.
48. Stout, A.K., Raphael, H.M., Kanterewicz, B.I., Klann, E., and Reynolds, I.J., Glutamate-induced neuron death requires mitochondrial calcium uptake, *Nat. Neurosci.*, 1, 366, 1998.
49. Sengpiel, B., Dreis, E., Kriegelstein, J., and Prehn, J.H., NMDA-induced superoxide production and neurotoxicity in cultured rat hippocampal neurons: Role of mitochondria, *Eur. J. Neurosci.*, 10, 1903, 1998.
50. Adjilore, O.A. and Sapolsky, R.M., Application of silicon microphysiometry to tissue slices: Detection of metabolic correlates of selective vulnerability, *Brain Res.*, 752, 99, 1997.
51. Zhu, P.J. and Krnjevic, K., Persistent block of CA1 synaptic function by prolonged hypoxia, *Neuroscience*, 90, 759, 1999.
52. Ozetecan, T., Kocak-Toker, N., and Aykag-toker, G., *In vitro* effects of peroxynitrite on human spermatozoa, *Andrologia*, 31, 195, 1999.

53. Violi, F., Marino, R., Milite, M.T., and Loffredo, L., Nitric oxide and its role in lipid peroxidation, *Diabetes/Metab. Res. Rev.*, 15, 283, 1999.
54. Virag, L., Scott, G.S., Antal-Szalmás, P., O'Connor, M., Ohshima, H., and Szabo, C., Requirement of intracellular calcium mobilization for peroxynitrite-induced poly (ADP-ribose) synthetase activation and cytotoxicity, *Molec. Pharmacol.*, 56, 824, 1999.
55. Bockowski, J., Lisdero, C.L., Lanone, S., Samb, A., Carreras, M.C., Boveris, A., Aubier, M., and Poderoso, J.J., Endogenous peroxynitrite mediates mitochondrial dysfunction in rat diaphragm during endotoxemia, *FASEB J.*, 13, 1637, 1999.
56. Supinski, G., Stotan, D., Callahan, L.A., Nethery, D., Nosek, T.M., and DiMarco, A., Peroxynitrite induces contractile dysfunction and lipid peroxidation in the diaphragm, *J. Appl. Physiol.*, 87, 743, 1999.
57. Distasi, A.M., Mallozzi, C., Macchia, G., Petrucci, T.C., and Minetti, M., Peroxynitrite induces tyrosine nitration and modulates tyrosine phosphorylation of synaptic proteins, *J. Neurochemistry*, 93, 927, 1999.
58. Yu, Z.F., Bruce-Keller, A.J., Goodman, Y., and Mattson, M.P., Uric acid protects neurons against excitotoxic and metabolic insults in cell culture and against focal ischemic brain injury *in vivo*, *J. Neurosci. Res.*, 53, 613, 1998.
59. Gunasekar, P.G., Borowitz, J.L., and Isom, G.E., Cyanide-induced apoptosis involves NMDA receptor-mediated oxidative stress and NF- κ B linked activation of caspase-3 protease, submitted for publication.
60. Fujiwara, N., Higashi, H., Shimoji, K., and Yoshimura, M., Effects of hypoxia on rat hippocampal neurons *in vitro*, *J. Physiol.*, 384, 131, 1987.
61. LeBlond, J. and Krnjevic, K., Hypoxic changes in hippocampal neurons, *J. Neurophysiol.*, 62, 1, 1989.
62. Haddad, G.G. and Donnelly, D.F., O₂ deprivation induces a major depolarization in brain stem neurons in the adult but not in the neonatal rat, *J. Physiol.*, 429, 411, 1990.
63. Wind, T., Prehn, J.H., Peruche, B., and Kriegstein, J., Activation of ATP-sensitive potassium channels decreases neuronal injury caused by chemical hypoxia, *Brain Res.*, 751, 295, 1997.
64. Latha, M.V., Borowitz, J.L., Yim, G., Kanthasamy, A., and Isom, G.E., Plasma membrane hyperpolarization by cyanide: Role of potassium channels, *Archiv. Toxicol.*, 68, 37, 1994.
65. Koyama, S., Jin, Y., and Akaike, N., ATP-sensitive and Ca²⁺-activated K⁺ channel activities in the rat locus coeruleus neurons during metabolic inhibition, *Brain. Res.*, 828, 189, 1999.
66. Yang, J.J., Chou, Y.C., Lin, M.T., and Chiu, T.H., Hypoxia-induced differential electrophysiological changes in rat locus coeruleus neurons, *Life. Sci.*, 61, 1763, 1997.
67. Zhu, P.J. and Krnjevic, K., Adenosine release mediates cyanide-induced suppression of CA1 neuronal activity, *J. Neurosci.*, 17, 2355, 1997.
68. Maire, J., Medilanski, J., and Straub, R., Release of adenosine, inosine, and hypoxanthine from rabbit non-myelinated nerve fibers at rest and during activity, *J. Physiol.*, 357, 67, 1984.
69. Kurbat, J., Buchanan, R., Wolff, S. and Yoon, K. W., Cyanide mediated adenosine release from rat hippocampal neurons, *Soc. Neurosci.*, Abstr. 19, 1961.
70. Green, R. and Haas, H., Adenosine actions on CA1 pyramidal neurons in rat hippocampal slices, *J. Physiol.*, 366, 119, 1985.
71. Trussel, L. and Jackson, M., Dependence of an adenosine-activated potassium current on a GTP-binding protein in mammalian central neurons, *J. Neurosci.*, 7, 3306, 1987.
72. Hammerstrom, A.K. and Gage, P.W., Inhibition of oxidative metabolism increases persistent sodium current in rat CA1 hippocampal neurons, *J. Physiol.*, 510, 935, 1998.

73. Friedman, J.E. and Haddad, G.G., Anoxia induces an increase in intracellular sodium in rat central neurons *in vitro*, *Brain Res.*, 663, 329, 1994.
74. Haigney, M.C., Lakatta, E.G., Stern, M.D., and Silverman, H.S., Sodium channel blockade reduces hypoxic sodium loading and sodium-dependent calcium loading, *Circulation*, 90, 391, 1994.
75. Jiang, S., Liu, Z., and Zhuang, X., Effect of procaine hydrochloride on CN intoxication and its effect on neuronal calcium in mice, *Toxicol. Appl. Pharmacol.*, 150, 32, 1998.
76. Baskin, S.I., Wilkerson, G., Alexander, K., and Blitstein, A.G., *Clinical and Experimental Toxicology of Cyanides*, Ballantyne, B. and Marrs, T. C., Eds., IOP Publishing Ltd., Bristol, England, 1987, 138.
77. Kondo, R.P., Apstein, C.S., Eberli, F.R., Tillotson, D.L. and Suter, T.M., Increased calcium loading and inotropy without greater cell death in hypoxic rat cardiomyocytes, *Am. J. Physiol.*, 275, H2292, 1998.
78. Elliott, A., Smith, G., Eisner, D., and Allen, D., Metabolic changes during ischemia and their role in contractile failure in isolated ferret heart, *J. Physiol.*, 454, 467, 1992.
79. Kupriyov, V., Yang, L., and Deslauriers, R., Cytoplasmic phosphates in $\text{Na}^+ - \text{K}^+$ balance in KCN-poisoned rat heart: a ^{87}Rb - ^{23}Na - and ^{31}P -NMR study, *Am. J. Physiol.*, 270, H1303, 1996.
80. Smith, G., Donoso, P., Bauer, C., and Eisner, D., Relationship between intracellular pH and metabolite concentrations during metabolic inhibition in isolated ferret heart, *J. Physiol.*, 492, 11, 1993.
81. Shanbaky, N. and Borowitz, J., Effect of pH on the response of adrenal medulla to various agents, *J. Pharmacol. Exp. Ther.*, 207, 998, 1978.
82. Maduh, E., Borowitz, J., Turek, J., Rebar, A., and Isom, G., Cyanide-induced neurotoxicity: calcium mediation of morphological changes in neuronal cells, *Toxicol. Appl. Pharmacol.*, 103, 214, 1990.
83. Lee, J. and Allen, D., Mechanisms of acute ischemic contractile failure of the heart: Role of intracellular calcium, *J. Clin. Invest.*, 88, 361, 1991.
84. Stewart, L., Deslauriers, R., and Kupriyanov, V., Relationships between cytosolic [ATP], [ATP]/[ADP] and ionic fluxes in the perfused rat heart, a ^{31}P , ^{23}Na , ^{87}Rb NMR study, *J. Mol. Cell Cardiol.*, 26, 1377, 1994.
85. Adler, M., Lebeda, F., Kaufmann, F., and Deshpande, S., Mechanism of action of sodium cyanide on rat diaphragm muscle, *J. Appl. Toxicol.*, 19, 411, 1999.
86. Okazaki, I.J. and Moss, J., Characterization of glycosylphosphatidyl inositol-anchored, secreted and intracellular vertebrate mono-ADP-ribosyltransferases, *Ann. Rev. Nutrition*, 19, 485, 1999.
87. Moss, J. and Vaughan, M., Activation of toxin ADP-ribosyltransferases by eukaryotic ADP-ribosylation factors, *Molec. Cell Biochem.*, 193, 153, 1999.
88. Mayer-Kuckuk, P., Ullrich, O., Ziegler, M., Grune, T., and Schweiger, M., Functional interaction of poly (ADP-ribose) with the 20S proteasome *in vitro*, *Biochem. Biophys. Res. Comm.*, 259, 576, 1999.
89. Stout, A.K. and Woodward, J.J., Mechanism for nitric oxide's enhancement of NMDA-stimulated [^3H] norepinephrine release from rat hippocampal slices, *Neuropharmacology*, 34, 923, 1995.
90. Lee, Y.J. and Shacter, E., Oxidative stress inhibits apoptosis in human lymphoma cells, *J. Biol. Chem.*, 274, 19792, 1999.
91. Masmoudi, A., Mandel, P., and Maluiya, A., Unexpected stimulation of mitochondrial ADP ribosylation by CN, *FEBS Lett.*, 237, 150, 1988.

92. Tsuyama, S., Fujita, H., Hijikata, R., Okamoto, H., and Takanaks, S., Effects of mono-ADP-ribosylation on cytoskeletal actin in chromaffin cells and their release of catecholamine, *Int. J. Biochem. Cell Biol.*, 31, 601, 1999.
93. Jones, D.H., Bax, B., Fensome, A., and Crockcroft, S., ADP ribosylation factor 1 mutants identify a phospholipase D effector region and reveal that phospholipase D participates in lysosomal secretion but is not sufficient for recruitment of coatamer 1, *Biochem. J.*, 341, 185, 1999.
94. Eliasson, M., Samper, K., Mandir, A., Hurn, P., Traystman, R., Bao, J., Peiper, A., Wang, Z., Dawson, T., Snyder, S., and Dawson, V., Poly (ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia, *Nat. Med.*, 3, 1089, 1997.
95. Venihaki, M., Gravanis, A., and Margioris, A., Opioids inhibit dopamine secretion from PC12 rat pheochromocytoma cells in a naloxone-reversible manner, *Life Sci.*, 58, 75, 1996.
96. Anderson, R. and Harland, W., *Forensic Toxicology*, Oliver, J. S., Ed., Droon Helan, London, 1989, 289.
97. Maehly, A. and Swensson, A., Cyanide and thiocyanate levels in blood and urine of workers with low grade exposure to CN, *Int. Arch. Arbeitsmed.*, 27, 195, 1970.
98. Moncala, S., Palmer, R.M.J., and Higgs, E.H., Nitric oxide: Physiology, pathophysiology and pharmacology, *Pharmacol. Rev.*, 43, 109, 1991.
99. Westley, J., *Cyanide in Biology*, Vennesland, B., Conn, E., Knowles, C., Westley, J., and Wissing F., Eds., Academic Press, New York, 1981, 61.
100. Sun, P., Borowitz, J., Kanthasamy, A., Kane, M., Gunasekar, P., and Isom, G.E., Antagonism of cyanide toxicity by isosorbide dinitrate: Possible role of nitric oxide, *Toxicology*, 104, 105, 1995.
101. Moore, S., Norris, J., Ho, I., and Hume, L., The efficacy of α -ketoglutaric acid in the antagonism of cyanide intoxication., *Toxicol. Appl. Pharmacol.*, 82, 44, 1986.