Further Investigations Regarding the Toxicity of Sodium Nitroprusside

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Sodium nitroprusside is a valuable vasodilator, but its use has been curtailed because of numerous reports that, in the presence of blood, nitroprusside decomposes with release of toxic cyanide. We have examined the release of cyanide in terms of the known chemistry of nitroprusside and suggest that photochemical decomposition of nitroprusside and (or) its metabolism in vivo invalidates the analytical procedure used by previous workers. We also present evidence for the stability of nitroprusside in blood, based on 13C nuclear magnetic resonance studies.

**Additional Keyphrases:** cyanide · nuclear magnetic resonance · analytical error

Sodium nitroprusside (Na2[Fe(CN)5NO]) is a valuable vasodilator for use in hypertensive emergencies, induced hypotension, and the management of myocardial infarction. However, its use has been curtailed because of reports, based on in vitro and in vivo experiments, that it decomposes in blood, releasing toxic cyanide (1). However, the method that was used for detecting cyanide (2) cannot distinguish between cyanide released from photolysed nitroprusside (some of which will always be produced during the assay) and free cyanide, and we thus consider it unsuitable. However, our criticism of the analytical procedures should not be taken as an assertion that cyanide is not released from nitroprusside in blood; further evidence for this must be presented.

From a chemical point of view, the decomposition of nitroprusside to cyanide is surprising because all cyanoferate complexes have very high formation constants.3 Indeed, the administration of iron(II) sulfate has been used as an antidote to cyanide poisoning for that reason. In an earlier study (4) we could find no evidence for cyanide release from nitroprusside in blood by the use of a cyanide-sensitive electrode; however, the need to use a sample pH of 12 to make the electrode responsive renders that technique far from ideal. A better way to approach the problem of the stability of nitroprusside would be to use a completely non-intrusive analytical procedure, simultaneously sensitive to nitroprusside and free cyanide. Nuclear magnetic resonance (NMR) spectroscopy with 13C has these characteristics and allows essentially continuous monitoring of the progress of the reaction. We now report the results of a study involving this novel analytical procedure. Normally, NMR spectra are obtained from naturally occurring 13C, which is present in only 1% abundance; this means the use of concentrated solutions and long accumulation times. However, the situation is dramatically changed if the abundance of 13C is enhanced.

**Materials and Methods**

Sodium [13C]cyanide was purchased from Merck, Sharp & Dohme and sodium nitroprusside in which 90% of the carbon atoms were 13C was prepared according to Butler et al. (5). Blood, sampled from one of us (A.R.B.), was treated with EDTA and the assay was commenced within 8 h of collection. Deuterium oxide (D2O), 0.2 mL, was added to 2.0 mL of the blood sample to provide an NMR lock signal. Na2[Fe(13C)5NO] (3 mg) was then added, and the sample was placed in the NMR spectrometer. The NMR spectra were obtained at 35 °C with a high-field (8.46 T, 90.56 MHz) instrument (Model WH-360; Bruker Instruments, Coventry, U.K.). The blood/nitroprusside mixture was kept in the spectrophotometer for 14 h and, during this time, the blood completely coagulated—this had no adverse effect on detection of nitroprusside or cyanide in the sample by running spectra at hourly intervals. The first spectrum was obtained with an accumulation time of 5 min, the last with an accumulation time of 14 h.

We also recorded the spectra of Na2[Fe(13C)6NO] (1.5 g/L) in phosphate buffer containing 10% (by vol) 1H2O and of Na2[13C] in blood (1.3 g/L) containing 10% (by vol) 1H2O.

**Results and Discussion**

With the high-field NMR spectrometer we could observe the very characteristic spectrum of the 13C-enriched nitroprusside ion (5, 6) (a large doublet centered at 134.4 ppm and a quintet centered at 132.4 ppm) at a concentration of 5 mmol/L (1.5 g/L) in phosphate buffer (pH 7.20), after an accumulation time of only 5 min (Figure 1). When the labeled nitroprusside was dissolved in whole human blood at the same concentration, the same spectrum was observed. This spectrum, recorded at hourly intervals, remained unchanged during 14 h (Figure 2). The concentration of nitroprusside we used was very much higher than that used in clinical practice for the control of blood pressure and also higher than that used in previous in vitro incubation experiments, and we do not wish to make any unwarranted deductions from the constancy of this spectrum. What is more significant is the absence of a signal due to 13CN−, which would have been present had the nitroprusside decomposed. In a separate experiment, addition of sodium [13C]cyanide to a blood sample gave a singlet NMR peak with a chemical shift of 121.0 ppm, readily distinguishable from that of the cyanogen groups of nitroprusside. With the blood/nitroprusside sample for which the spectrum had been accumulated over 14 h, giving it an extremely favorable signal-to-noise ratio, there was no sign whatsoever of a peak at 121.0 ppm. However, had only 3% of the added nitroprusside decomposed, 750 μmol of cyanide per liter would have been produced, an amount readily observable in a spectrum with such a long accumulation time. The sensitivity to free

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3 ββ for hexacyanoferate(II) and hexacyanoferate(III) are defined as follows:
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5 and have values (3) of about 1046 in the case of Fe(II) and 1043 in the case of Fe(III).
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490 CLINICAL CHEMISTRY, Vol. 33, No. 4, 1987
cyanide is enhanced by the fact that the two split signals in nitroprusside generate a single unsplitted signal in free cyanide. Vesey et al. (7) claimed that 0.3 mmol of nitroprusside per liter in blood released about 620 μmol of cyanide per liter after incubation for 3 h, and Spiegel and Kucera (8) reported that 850 μmol of cyanide per liter was observed on incubation of nitroprusside with blood. Thus the sensitivity of our non-intrusive method is such that we could have detected cyanide at the concentrations found by other workers who used a different analytical procedure. Our work cannot be said to simulate the nitroprusside concentrations used in clinical practice; rather, it addresses the evidence for cyanide release from nitroprusside based on in vitro blood-incubation experiments.

Why is our result inconsistent with that obtained by the analytical procedure of previous workers? In that procedure the blood sample, after incubation with sodium nitroprusside in vitro, is acidified to convert CN− to HCN. The volatile HCN, blown from the sample by bubbling nitrogen through it, is trapped in sodium hydroxide solution, in which the concentration of cyanide is then determined colorimetrically (2). In this method for detecting cyanide in the presence of nitroprusside it is assumed that nitroprusside itself is not decomposed to HCN on acidification and the passage of nitrogen. This assumption is, indeed, correct but in practice the situation is a little more complicated. Nitroprusside very readily undergoes photolysis (9, 10) to aquapentacyanoferrate(2−):

\[ \text{H}_2\text{O} + [\text{Fe(CN)}_5\text{NO}]^{2−} \xrightarrow{\text{hv}} [\text{Fe(CN)}_5\text{H}_2\text{O}]^{2−} + \text{NO} \]

which does decompose to HCN on acidification and the passage of nitrogen. In a previous paper (4) we pointed out that this casts doubt on the quantitative aspects of previous analyses, and elsewhere (11) we discussed the chemical principles behind the decomposition of aquapentacyanoferrate(2−). The photolytic degradation of nitroprusside commences immediately when the material goes into solution, but the extent of this reaction depends on the amount of light the sample receives adventitiously during normal laboratory handling. For in vitro experiments involving incubation with blood there is little possibility of photolysis after mixing, because of the opaque nature of blood. However, photolysis will occur before nitroprusside is mixed with blood and during analysis, a procedure that lasts several hours. Because the amount or intensity of exposure to light usually is not recorded in accounts of experiments with nitroprusside, we cannot assess the extent of photolysis, but some will always occur. And, as stated before, the usual analytical procedure cannot distinguish between free cyanide and photolysed nitroprusside. Here, an analytical method having none of the uncertainties associated with the removal of HCN from an acidified solution yields results that fail to confirm the widely held view that, in vitro, blood will effect decomposition of nitroprusside to cyanide.

Our experiments described above parallel previous in vitro work, but in vivo studies pose an additional analytical problem. Aquapentacyanoferrate(2−) decomposes in acid solution during analysis whereas nitroprusside does not, because the former contains iron(III) and the latter iron(II). All iron(III) complexes contain labile cyano-ligands and will be degraded under the conditions of analysis to release HCN. When nitroprusside acts hypotensively in vivo it must be metabolized. The chemistry of this process is unknown,
but if metabolism results in formation of an iron(III) complex with cyano-ligands then it is inevitable that results by the analytical procedure generally used would suggest that free cyanide is present. Until the chemistry of the hypotensive action of nitroprusside is known, only analytical procedures that can distinguish unambiguously between free cyanide and a labile iron(III) cyano-complex are valid. Such methods have not been used in previously reported work and this, together with the uncertain incidence of photolysis during analysis, leads us to conclude that, to date, there is no certain evidence that nitroprusside decomposes in blood to give free cyanide, a view consistent with our $^{13}$C NMR data.

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