

Title: THE DECOMPOSITION OF SODIUM NITROPRUSSIDE BY BLOOD COMPONENTS

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Introduction. The vasodilator sodium nitroprusside (NP) is characterized by a rapid onset, a rapid (2-5 minute) return of pre-infusion blood pressures after cessation, and low plasma concentrations (about 100 nanomolar). Most studies to date on NP metabolism have involved using NP concentrations several orders of magnitude higher than the pharmacologically active concentration and have looked indirectly at NP degradation by following cyanide production. There has only recently been one method of direct NP detection sensitive down to micromolar concentrations. We have prepared carbon 14 labeled NP and studied its stability in biological solutions at 100 nM.

Methods. NP was prepared from ferrous chloride, sodium nitrite, and carbon 14 labeled potassium cyanide. It was purified chromatographically and purity was confirmed by ion-pair HPLC, gel ion exchange chromatography, and chemical reactivity. Specific activity is 55.6 mCi/mmol of CN or 278 mCi/mmol of NP. Heparinized human blood, pooled from several patients was obtained from the arterial blood gas laboratory in our operating suite, within 4 hours of collection. Samples were prepared as indicated, aliquots of NP (to 100 nM) added at time 0, and incubated at room temperature or 37 degrees Celsius in the dark. At various times, typically 0, 10, 20, 30, and 60 minutes, samples were withdrawn, centrifuged in the case of blood, and 0.3-0.5 ml of the liquid phase applied to a small (bed volume=0.1 ml) column of QAE Sephadex equilibrated in 0.01 M sodium phosphate pH 7.5. The column was then washed with 0.2 ml of the same buffer. The eluted volume from the column was counted as one sample, and the QAE resin was counted as another. Under these conditions it was established that NP binds tightly to the resin and cyanide is eluted. Unstable purple complexes formed by the reaction of NP with sulfides or sulfhydryls and other unidentified intermediates of those reactions also eluted from the columns.

Results. NP decay could be clearly followed and exhibited apparent first order kinetics. In samples not containing erythrocytes (RBCs) the kinetics obtained by following NP (resin bound cpm) agreed with the kinetics obtained following cyanide production (eluted cpm). Samples containing RBCs showed only a gradual rise in eluted cpm presumably due to sequestering of cyanide by hemoglobin in the RBC. Cell-bound radioactivity was not followed. The loss of NP from the liquid phase of solutions containing RBCs showed decay kinetics similar qualitatively and quantitatively to solutions not containing RBCs. Solutions tested were:

1. fresh whole blood, tested at 20 and 37 degrees.
2. whole blood at 20 degrees after sitting refrigerated for 24 hours.

3. plasma, tested at 20 and 37 degrees.

4. RBCs, at 37C, washed twice in a phosphate buffered (pH 7.5) normal saline, and resuspended to the original blood volume.

5. free hemoglobin (Hb) at 37 degrees prepared, under nitrogen, from RBCs hemolysed in .01 M Na phosphate buffer pH 7.5. Solutions were centrifuged and diluted to 8 times the original blood volume. Spectrophometric analysis before and after the addition of carbon monoxide and subsequent dithionite showed the Hb to be essentially all reduced oxyhemoglobin. Hb passed freely through the QAE resin.

6. whole blood at 20 degrees plus 80 mM NaNO₂ and 30 mM KCN to convert heme to cyanmetheme.

Table 1 shows the results expressed as half lifes and standard errors obtained from the first order decay curve by linear regression (ln cpm vs time).

Discussion. The results show that NP is degraded at pharmacological concentrations by the components of blood. Both the plasma and RBC fractions are active in decomposing NP, and the rate of decomposition is temperature-dependent. However, the rate of decomposition by blood is far too slow to account for the short biological half life of NP. Estimating 2 minutes for its biological half life, less than 10% of NP degradation would be caused by blood components.

After sitting for 24 hours blood causes more decomposition of NP. This is likely due in part to cell lysis since it is shown that a supernatant of hemolysis is more active. It is noteworthy, though, that the 8 fold diluted hemolysis supernatant produces a longer half life than whole blood. Extrapolation gives a half life for undiluted supernatant of >6 min. It appears that plasma contains activity towards NP beyond that which can be explained by small amounts of hemolysis. The same also appears to be true for the intact cellular components of blood. The activity in all phases of blood is completely blocked by treatment with nitrite and cyanide. This treatment converts heme to cyanmetheme but at these concentrations may also inactivate -SH, -NH₂ or other potentially reactive groups.

References. 1. Leeuwenkamp, et. al.: In vitro degradation of NP in relation to in vivo decomposition. Int.J.Pharm. 33:1 (1986).

Table 1. Half life (in minutes) of NP decay.

Materials	half-life	std. err.
plasma 20C	231	5
plasma 37C	104	2
blood 20C	37	0.8
blood 37C	22	0.2
blood 1 day old, 20C	21	0.3
blood,nitrite,cyanide	no decomp.	
hemolysis supernatant	51	0.3
washed cells	32	0.2