J. Pharm. Pharmacol. 1987, 39: 936–938
Communicated May 7, 1987

The effect of hydroxocobalamin on the nitroprusside-induced relaxation of rat aortic preparations

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Hydroxocobalamin (HOCb) when mixed with sodium nitroprusside (SNP) in a 10:1 or 1:1 molar ratio had no significant effect on the relaxation of noradrenaline-precontracted rat aortic rings. However, the addition of HOCb did prolong the time taken for the relaxant response to occur. The time taken for a 0·34 μM SNP dose to exert 50% of its relaxant effect was increased by about 120 and 35% with 10:1 and 1:1 HOCb/SNP molar ratio mixtures, respectively. A 0·5:1 HOCb/SNP molar ratio mixture caused no prolongation. In incubations with [^{14}C]SNP, it was found that mixing with equimolar, or greater, amounts of HOCb, reduced aortic tissue uptake of radioactivity. The results support the hypothesis that both SNP alone and SNP/HOCb complexes breakdown extracellularly to release the same amount of active species, but that in the case of the complexes the active agent is released at a slower rate.

Sodium nitroprusside (SNP) is a potent, short-acting vasodilator with an established clinical usage (Kreye 1980). A disadvantage of the drug, however, is that large doses may be degraded in the body to produce potentially toxic amounts of cyanide (Kreye 1980). One suggested way in which SNP-induced cyanide toxicity could be treated or avoided (Cottrell et al 1978) is by the use of hydroxocobalamin (HOCb), which combines with cyanide to form the relatively non-toxic cyanocobalamin. However, the use of HOCb suffers from a number of disadvantages, including the fact that it may alter the pharmacological response to SNP. This was shown in anaesthetized rats by Hewick et al (1987), who demonstrated that when HOCb and SNP were mixed in a 10:1 or 1:1 molar ratio and given in intravenous bolus doses, the depressor response to SNP was prolonged, although the degree of blood pressure lowering was unaffected. Since the presumed site of action of SNP is the vascular smooth muscle, it was decided to examine further the mechanism of the HOCb/SNP interaction, using rat aortic preparations.

Materials and methods

Male Sprague-Dawley rats (200–400 g) were killed by cervical dislocation, and transverse ring segments (about 4 mm in length) prepared from the thoracic aortae. Each segment was mounted between two intraluminal steel wires essentially according to Towart (1984) in a 10 mL organ bath containing physiological Ringer solution, maintained at 37 °C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The tissues were allowed to equilibrate (under a 1 g tension) for 90 min. Then a maximal dose of noradrenaline (4·86 μM) was added to maintain a tonic contraction, followed by cumulative doses of SNP or an HOCb/SNP mixture. Pairs of aortic rings (one segment receiving the SNP alone and one receiving the mixture) from each rat were tested at the same time. Muscle tension was recorded using 25 g strain-gauge transducers, an MX2P preamplifier and a Multitrace 2 chart recorder (Ormed Ltd, Welwyn Garden City, Herts, UK).

In separate experiments, the time taken for relaxation to occur with SNP or HOCb/SNP mixtures was assessed. Noradrenaline-precontracted tissue was used as before. A dose (0·34 μM) of SNP (either alone or mixed with HOCb) was employed which produced about 95% relaxation. Again pairs of aortic ring segments were used, one segment receiving SNP alone, the other an HOCb/SNP mixture. The HOCb/SNP mixtures (expressed as molar ratios) were added in triplicate in the following order; 10:1, 1:1, 0·5:1, 1:1, 0·5:1, 10:1, 0·5:1, 10:1, 1:1, to allow for any tissue sensitivity changes during the course of the experiment. After each relaxant response, the tissue was washed and allowed to re-equilibrate for about 10 min before being precontracted again with noradrenaline. The parameter measured was the time taken for the 0·34 μM SNP dose (alone or mixed with HOCb) to produce 50% of its relaxant effect.

The uptake of SNP into the aortic tissue was measured using the ^{14}C-labelled material (61 μCi mmol^{-1}) synthesized as described by Hewick et al (1987). Four aortic ring segments were taken from each rat with two experimental and two control determinations being carried out. Each segment was incubated in a 10 mL glass vial containing noradrenaline (4·86 μM), [^{14}C]SNP (3·4 mM, 0·205 μCi mL^{-1}), HOCb (for incubation of 'mixtures' only) with Ringer solution (saturated with 95% O_2: 5% CO_2) added to a volume of 1 mL. The incubations were carried out in a shaking incubator (70 oscillations min^{-1}) at 37 °C. After incubation, each aortic segment was removed from the vial and washed with a solution which was identical to the incubation fluid, except that it contained only

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unlabelled SNP. The tissue was then blotted dry and its radioactive content determined by standard liquid scintillation counting methods (Griffiths et al 1984). Radioactivity was expressed as SNP equivalents.

In all experiments where HOCb, noradrenaline and SNP were used in aqueous solutions they were protected from light.

All experimental results are means ± s.e. Comparisons were made using Student's paired *t*-test, with a probability of <0.05 being taken as significant.

Results

HOCb added to SNP in a 10 : 1 or 1 : 1 molar ratio had no significant effect on the extent to which a range of SNP doses relaxed the aortic tissue (Fig. 1A and B). However, it was noticed that the addition of HOCb did prolong the time taken for each response to occur. This effect for the 0·34 μM SNP dose is shown in Table 1. The time taken for this dose to exert 50% of its relaxant effect was increased by 117 and 34% with 10 : 1 and 1 : 1 HOCb/SNP molar ratio mixtures, respectively. A 0·5 : 1 HOCb/SNP molar ratio mixture caused no significant prolongation.

The uptake of [^{14}C]SNP-derived radioactivity by aortic rings is shown in Table 2. It is seen that greater uptake occurred after 600s of incubation than after 30 s, for both SNP alone and the HOCb/SNP mixtures. It is also seen that, despite variations in control values, particularly with the 30 s incubations, HOCb mixed with SNP in a 10 : 1 or 1 : 1 molar ratio decreased uptake of radioactivity by 20–33% at both 30 and 600 s incubation times. Mixing HOCb with SNP in a 0·5 : 1 ratio had no effect on uptake.

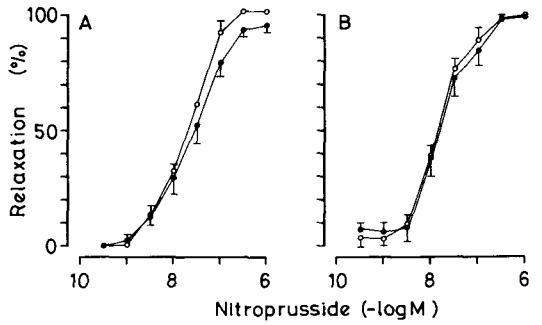

FIG. 1. The relaxation of rat aortic rings precontracted with noradrenaline (4·86 μM) by the cumulative addition of sodium nitroprusside either alone (open symbols) or mixed with hydroxocobalamin (closed symbols). Panels A and B refer, respectively, to the use of 10 : 1 or 1 : 1 molar ratio mixtures of hydroxocobalamin : nitroprusside. The data points are means ± s.e. (n = 5).

Table 1. The effect of hydroxocobalamin on the time course of the nitroprusside-induced relaxation of rat aorta.

Molar ratio of HOCb : SNP mixture	Time taken (s) for 0·34 μM nitroprusside to produce '50% relaxation'		
	SNP alone	Mixture	% prolongation
10 : 1 (4)	17·9 ± 1·6	38·9 ± 3·7	117*
1 : 1 (4)	18·8 ± 2·5	25·1 ± 4·2	34*
0·5 : 1 (4)	19·5 ± 2·0	21·0 ± 2·7	8

Pairs of aortic ring segments from the same rat were precontracted with noradrenaline (4·86 μM) as described in 'Materials and methods' and relaxation was produced by the addition of 0·34 μM nitroprusside either alone or mixed with hydroxocobalamin. The figures in parentheses indicate the numbers of rats used. Means ± s.e. are given. Asterisks indicate a significant prolongation of the onset of relaxant response ($P < 0.05$).

Discussion

The results obtained in the present investigation in-vitro support those obtained by Hewick et al (1987) using an anaesthetized rat/blood pressure model. In both studies HOCb was found to prolong the onset of the SNP response without significantly altering its magnitude. The cause of the effect of HOCb on the SNP response is the complexation occurring between the two compounds, involving co-ordination between the cyanide ligands of the nitroprusside and the cobalt of the cobalamin (Butler et al 1986). Mixing HOCb and SNP in 10 : 1 and 1 : 1 molar ratios results in 2 : 1 and 1 : 1 HOCb-nitroprusside complexes respectively (Butler et al 1986).

In patients and whole-animal preparations, control blood pressure values are restored within a few minutes of stopping intravenous injection of SNP (Tuzel 1974; Hewick et al 1987). This is thought to be due to the rapid in-vivo breakdown of the drug which appears to be associated with its interaction with blood vessels, rather than any components of the blood (Leeuwenkamp et al 1986). It is also thought that such an interaction initiates the pharmacological response, in which the nitrosyl ligand of SNP is presumed to be the active species (Ignarro et al 1981). Current ideas on the mechanism of action of nitroprusside support an intracellular site of action involving the nitrosyl group reacting with guanylate cyclase (see Leeuwenkamp et al 1986).

The results of Hewick et al (1987) along with those of the present work, using SNP mixed with HOCb and showing that the magnitude of response is unaffected by complexation, suggest that both SNP alone and the SNP/HOCb complex release all (or the same amount) of the active species. However, the slower onset of response associated with the complex indicates a reduced rate of release of the active agent. Since it is improbable that the bulky HOCb/SNP complex could, to any significant extent, penetrate the arterial tissue,

Table 2. The effect of hydroxocobalamin on the uptake of [^{14}C]nitroprusside-derived radioactivity by rat aortic tissue.

Molar ratio of HOCb : SNP mixture	Uptake of [^{14}C]nitroprusside-derived radioactivity (nmol SNP-equivalent per mg wet weight tissue) after incubation for: 30 s			600 s		
	SNP alone	Mixture	% decrease	SNP alone	Mixture	% decrease
10 : 1 (5)	1·07 ± 0·11	0·75 ± 0·05	30*	1·64 ± 0·16	1·1 ± 0·10	33*
1 : 1 (9)	0·56 ± 0·06	0·45 ± 0·07	20*	1·25 ± 0·16	0·84 ± 0·06	33*
0·5 : 1 (6)	0·80 ± 0·1	0·70 ± 0·18	12	1·25 ± 0·23	1·23 ± 0·10	2

Aortic ring segments were incubated with noradrenaline (4·86 μM), [^{14}C]nitroprusside (3·4 mM, 0·205 μCi mL^{-1}) and hydroxocobalamin (incubation of 'Mixtures' only) at 37 °C. Comparisons between SNP alone and mixtures were made using 'pairs of tissues' prepared from the same rat. The figures in parentheses indicate the numbers of rats used. Uptake values are given as means ± s.e. Asterisks indicate a significant reduction of uptake ($P < 0·05$).

the most likely site of major degradation (and possible release of the active moiety) is the intimal surface of the blood vessel wall. The SNP alone, in the form of the polar nitroprusside ion, could also undergo major breakdown at this site.

The experiments showing that HOCb reduces the tissue uptake of [^{14}C]SNP-derived radioactivity support the hypothesis that the extracellular degradation of the HOCb/SNP complex and resultant production of the active species is slower than that for SNP alone. A discrepancy which should be noted is that the uptake of radioactivity was inhibited after 600 s as well as 30 s of incubation. Since by 600 s relaxation responses of the same magnitude would have been completed both by the HOCb/SNP complex and the SNP alone, it would have been expected that similar amounts of drug would have been taken up. However, precise comparisons between the tissue uptake and organ bath studies are not possible because, due to the low specific activity of the [^{14}C]SNP synthesized, 10 000 times higher concentrations of SNP had to be used in the former study. Furthermore, only the uptake of [^{14}C]SNP-derived radioactivity was measured and the extent to which this was associated with the uptake of the supposedly active nitrosyl species is not known. In spite of these limitations, since it is probable that the degradation process which produced [^{14}C]products also involves the release of the active species, the studies using [^{14}C]SNP do support the idea that the HOCb complexation reduces the rate of production and uptake of the active 'relaxant' agent.

The present findings can therefore be summarized as follows. Mixing SNP with HOCb (equimolar or molar excess) results in the production of an SNP/HOCb complex and a slower onset of action which is probably due to a slower release of the active agent. This slower release is suggested by the reduced tissue uptake of [^{14}C]SNP-derived radioactivity, if it is assumed that uptake of radioactivity reflects to some degree the uptake of the active species.

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