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Sodium nitroprusside: pharmacological aspects of its interaction with hydroxocobalamin and thiosulphate

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Hydroxocobalamin (HOCb), when mixed with sodium nitroprusside (SNP) in a 10 : 1 or 1 : 1 molar ratio and injected (i.v.) into the anaesthetized rat, prolonged the depressor response to SNP by 25–50%, but did not affect the degree of blood pressure lowering. Both the 'onset' and 'offset' components of the response were prolonged. Injecting [^{14}C]SNP along with a 10-fold molar excess of HOCb resulted in a 2- to 3-fold elevation of plasma radioactivity which was maintained during the first 10 min of a 40 min experimental period. These effects of HOCb on the pharmacodynamics and pharmacokinetics of SNP are probably due to complex formation between the two compounds. Sodium thiosulphate (ST) added to SNP (12 : 1 molar ratio) had no effect on the depressor response to SNP. This mixing of ST and SNP had a less-marked influence on the plasma [^{14}C]SNP-derived radioactivity than occurred with HOCb. There was no initial elevation of radioactivity, but the levels were raised by 50–60% at 4, 6 and 10 min. Since the depressor response to SNP was unaffected by ST, it is presumed that the higher concentrations of radioactivity were due to inactive degradation products rather than the active species itself.

Sodium nitroprusside (SNP) is a potent short-acting vasodilator with an established use in the treatment of hypertensive emergencies, heart failure, and in the deliberate induction of hypotension during anaesthesia (Kreye 1980). With a formula of Na_2-$[Fe(CN)_5NO].2H_2O$, the cyanide ligand accounts for 44% by weight of the molecule. Although the extent to which this cyanide is released in the blood has been disputed (Bisset et al 1981; Smith et al 1982), it is presumed to be released in the body and to account for the acute toxicity of SNP (Cole 1978; Ivankovich et al 1978; Kreye 1980). Such toxicity may be reduced by the administration of sodium thiosulphate (ST) (Schulz et al 1982; Pasch et al 1983). The exogenous ST replenishes depleted endogenous thiosulphate stores used up by initial rhodanese (thiosulphate : cyanide sulphur transferase EC2.8.1.1)-mediated cyanide detoxication (Baumeister et al 1975). To retain the pharmacological benefits of SNP but remove the possibility of cyanide toxicity, a 'mixed infusion' technique has been advocated (Schulz 1984) in which ST and SNP in a 12 : 1 molar ratio) are mixed in the infusion fluid prior to injection.

Hydroxocobalamin (HOCb) has also been suggested for possible use in SNP-associated cyanide toxicity (Cottrell et al 1978), the cyanide combining with the HOCb to form the relatively non-toxic cyanocobalamin which is readily excreted. Since it was conceivable that a 'mixed infusion' of HOCb and SNP could be used clinically, and since ^{13}C-nuclear magnetic resonance spectroscopy has demonstrated a chemical interaction between HOCb and SNP (Butler et al 1986) we initiated studies to examine the pharmacodynamic and pharmacokinetic consequences of such an interaction. It was also decided to ascertain if there were any pharmacological consequences of mixing SNP and ST.

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MATERIALS AND METHODS

Chemicals

The HOCb was purchased from Sigma London (Poole, Dorset, UK). Sodium hexacyanoferrate(II) and ST were obtained from BDH Chemicals Ltd (Poole, Dorset, UK) and were of Analar grade. [^{14}C]Sodium hexacyanoferrate (2·94 mCi mmol^{-1}) was purchased from Amersham International (Amersham, Bucks, UK). Where aqueous SNP solutions were used, these were protected from light.

Preparation of [^{14}C]sodium nitroprusside

This was modified from the method of Seel (1965). [^{14}C]Sodium hexacyanoferrate (2·5 g, $Na_4Fe(CN)_6 10H_2O$, 50–70 μCi mmol^{-1}) was added to an ice-cold mixture of 2·25 mL nitric acid (S.G. 1·42) and water (1 mL) with stirring. More [^{14}C]hexacyanoferrate of the same specific activity (1·54 g) was added in portions over 4 h and then the mixture was allowed

to warm to room temperature over 1 h. After heating to 60 °C sodium carbonate (0·22 g) was added carefully in portions over 30 min. The mixture was then heated to 75 °C, more sodium carbonate (0·18 g) added, and stirred for 1 h at 60 °C. After addition of a mixture of methanol (10 mL) and water (3 mL) the solution was allowed to stand at 50 °C for 48 h in the dark under a gentle stream of nitrogen. Solid material was removed by filtration through a bed of Hyflo-Supercell (BDH Chemicals Ltd, Poole, Dorset, UK) and the filtrate concentrated on a rotary evaporator below 80 °C until crystals began to form. Just enough water was added to redissolve the crystals in the hot solution, which was set aside to crystallize. The small red needles were filtered off and dried between filter papers. More product was obtained by evaporation of the mother liquor and extracting the residue with warm methanol. The yield of [^{14}C]sodium nitroprusside (Na_2 [$Fe(C)_5$-NO].$2H_2O$) was 1·96 g, 6·58 mmol (79%) which had a specific activity of 61 μCi $mmol^{-1}$.

Animals

Male Sprague Dawley rats (450–650 g) maintained on a normal diet with free access to water were used.

Measurement of blood pressure and depressor response

The rats were anaesthetized with urethane (1·5 g kg^{-1} i.p.) and the rectal temperature maintained at 37 °C by means of a heat-lamp regulator device (Yellow Springs Instrument Co, Yellow Springs, OH). In each rat the right carotid artery and left jugular vein were cannulated for measurement of blood pressure and injection of drugs, respectively. The carotid cannula was connected to a blood pressure transducer (Elcomatic Ltd, UK) and blood pressure was monitored on a chart recorder (Ormed Ltd, Welwyn Garden City, UK). The venous cannula was connected to a 1·0 mL syringe. Drug solutions were made up in physiological saline, injected in a volume of 0·1–0·3 mL, and washed in with 0·1 mL saline.

Separate comparisons were made between SNP and a SNP/HOCb mixture, or SNP and a SNP/ST mixture. For each comparison a high (A) and low (B) dose of SNP were used along with a high (C) dose and low (D) dose of mixture. In each case the low dose was 25–50% of the high dose. During the comparisons each dose was given four times in a randomized order (ABCD, ACBD, CABD, BCDA) to allow for any sensitivity changes in the preparation during the course of the experiment. At the start of the experiment, the initial blood pressure of the rats used was about 100/80 mmHg (systolic/diastolic). The depressor response was assessed in the following ways; blood pressure lowering (the difference between the mean systolic pressure before drug addition and the lowest systolic pressure after drug addition, onset time (time between drug addition and maximum blood pressure lowering), 'offset' time (time between maximum blood pressure lowering and normalization of blood pressure). The time after normalization of blood pressure and injection of the subsequent dose was 1–2 min.

Measurement of plasma concentrations of [^{14}C]-sodium nitroprusside-derived radioactivity

The rats were anaesthetized and cannulated as previously described, the artery and vein being used for withdrawal of blood and injection of drugs, respectively. SNP (5 μmol^{-1} kg, 0·3 μCi^{-1} kg) was injected in physiological saline, either alone or with HOCb or ST and washed in with saline. Blood samples (0·4 mL) were withdrawn at 1, 2, 4, 6, 10, 20, 40 min and centrifuged (3000*g* for 10 min) to obtain plasma. Plasma samples (0·1 mL) were transferred to 5 ml NE260 scintillator (Nuclear Enterprises Ltd, Edinburgh, UK) for scintillation counting. Plasma radioactivity was expressed as SNP equivalents. Comparisons were made using two rats daily (one receiving SNP alone and one receiving a mixture of SNP and HOCb or ST). The experiments involving HOCb or ST were carried out several months apart.

Statistics

All experimental results are means ± s.e. Comparisons were made using Student's *t*-test, with the paired *t*-test being used for comparisons within the same animal. A probability of $<0·05$ was taken as significant.

RESULTS

The effect of hydroxocobalamin on sodium nitroprusside pharmacodynamics and plasma disposition

HOCb added to SNP in a 10 : 1 or 1 : 1 molar ratio, caused significant prolongations in both the onset and offset of the depressor response to maximal doses (60 or 200 nmol) of SNP but, as would be expected, had little effect on the degree of blood pressure lowering achieved (Table 1). However, a similar pattern was obtained with smaller and established submaximal doses of SNP (20 and 5 nmol) using a 10 : 1 molar ratio HOCb : SNP mixture. The higher submaximal dose (SNP alone or

Table 1. The effect of hydroxocobalamin on the depressor response to sodium nitroprusside.

Molar ratio of HOCb : SNP mixture	Dose of SNP (nmol)	Blood pressure lowering (mmHg) SNP alone	Mixture	% change	Onset time (s) SNP alone	Mixture	% change	Offset time (s) SNP alone	Mixture	% change
10 : 1	600	64·8 ± 8	68 ± 4	↑ 8	65 ± 7	100 ± 5	↑ 54*	362 ± 11	444 ± 14	↑ 23*
	200	56 ± 6	58 ± 6	↑ 4	47 ± 3	67 ± 3	↑ 43*	278 ± 9	332 ± 13	↑ 19*
1 : 1	600	41 ± 1	41 ± 1	0	62 ± 3	64 ± 10	↑ 3	368 ± 22	521 ± 45	↑ 42*
	200	36 ± 2	36 ± 2	0	45 ± 2	60 ± 5	↑ 33*	254 ± 8	339 ± 9	↑ 33*
0·5 : 1	600	38 ± 6	34 ± 4	↓ 11	47 ± 9	42 ± 12	↓ 11	398 ± 23	433 ± 25	↑ 9
	200	34 ± 5	33 ± 5	↓ 3	41 ± 7	41 ± 4	0	273 ± 25	288 ± 20	↑ 5

Intravenous bolus doses of SNP were given alone or as mixtures with HOCb. Each dose was administered four times in the order indicated in 'Materials and Methods'. The data are representative, one rat being used for each different HOCb : SNP mixture. Means ± s.e. are given (n = 4). Arrows indicate an increase/decrease compared with SNP alone and asterisks indicate that the difference is significant ($P < 0.05$).

mixture, respectively) in a typical experiment gave blood pressure lowering, onset and offset time values of 20 ± 0·3, 18 ± 0·9 mmHg; 26 ± 2, 50 ± 7 s; and 71 ± 4, 85 ± 4 s. The corresponding values for the lower dose were 16 ± 2, 14 ± 1 mmHg; 22 ± 2, 37 ± 3 s; 76 ± 4, 99 ± 7 s . Similar results in which the degree of blood pressure lowering was unaffected and the duration of response was significantly prolonged were given by two further rats. The increases in mean onset and offset times in the three rats for the 20 nmol SNP doses were 92, 83, 102 and 20, 16, 5% respectively. The corresponding values for the 5 nmol doses in the same rats were 68, 99, 102 and 30, 11, 42%, respectively.

HOCb added to SNP (600 or 200 nmol doses) in a 0·5 : 1 molar ratio had no significant effect on the depressor response to SNP (Table 1). Furthermore, HOCb alone had no hypotensive effect when administered in doses ranging from 50 to 6000 nmol (results not shown).

Addition of HOCb to the injection solution in a 10 : 1 HOCb : SNP molar ratio, resulted in an elevation of [^{14}C]SNP-derived radioactivity in the plasma. During the first 10 min the plasma elimination profiles for the SNP alone and the mixture were roughly parallel, with the concentrations of radioactivity associated with the latter being 2–3 times greater (Fig. 1a).

The effect of sodium thiosulphate on sodium nitroprusside pharmacodynamics and plasma disposition

ST added to SNP had no significant effect on the depressor response to SNP. In a rat given submaximal doses (12 and 6 nmol) of SNP either alone or as an ST : SNP mixture (12 : 1 molar ratio), the higher dose (SNP or misture, respectively) gave blood pressure lowering, onset and offset time values of 32 ± 2, 34 ± 3 mmHg; 22 ± 2, 23 ± 2 s; and 59 ± 3, 50 ± 4 s. The corresponding values for the lower dose were 21 ± 1, 25 ± 3 mmHg; 20 ± 1, 19 ± 1 s; 46 ± 7, 50 ± 4 s. Similar results were given by a further 3 rats.

Addition of ST to the injection solution to give a 12 : 1 ST : SNP molar ratio had a less marked

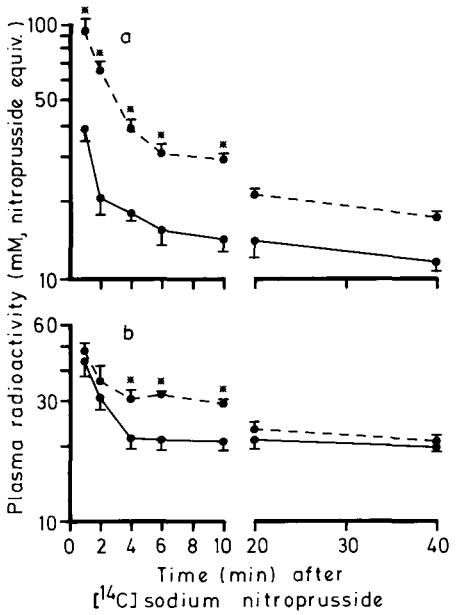

FIG. 1. The effect of mixing hydroxocobalamin (a) or thiosulphate (b) with [^{14}C]sodium nitroprusside on plasma radioactivity. The hydroxocobalamin or thiosulphate (10 or 12 fold molar excess, respectively) were mixed with the nitroprusside before injection. The nitroprusside dose was 5 μmol kg^{-1}, 0·3 μCi kg^{-1} (i.v.). The continuous and dashed lines correspond to 'nitroprusside alone' and 'mixture', respectively. The data points are means ± s.e. (n = 4 and 6 for hydroxocobalamin and thiosulphate experiments, respectively). Asterisks indicate a significant difference ($P < 0.05$) from nitroprusside alone.

influence on the plasma [^{14}C]SNP-derived radioactivity than HOCb. The main effect was an elevation of 50–60% for plasma radioactivity at 4, 6 and 10 min (Fig. 1b).

DISCUSSION

Preliminary experiments using [^{14}C]SNP, undertaken to examine the stability of SNP in blood and buffer suggested that SNP forms a complex with HOCb (Butler & McIntosh, unpublished observations). Further studies showed that mixing HOCb and SNP in 1:1 and 10:1 molar ratios resulted respectively in 1:1 and 2:1 HOCb-nitroprusside complexes. The use of high-field ^{13}C-nuclear magnetic resonance spectroscopy demonstrated that the formation of the complexes involved co-ordination between the cyanide ligand of the nitroprusside and the cobalt of the cobalamin (Butler et al 1986).

Mixing SNP with a molar excess of HOCb prolonged the depressor response in the anaesthetized rat by 30–70%, but appeared not to increase the degree of blood pressure lowering. The prolongation of response could be linked with a marked elevation of plasma [^{14}C]SNP-derived radioactivity when HOCb and SNP were injected together as a 10:1 molar ratio mixture. Since such a mixture results in the production of a 2:1 HOCb-nitroprusside complex (Butler et al 1986), it is apparent that such complexation tends to retain the injected SNP within the plasma space. If the rat plasma volume is assumed to be 40·4 mL kg^{-1} (Baker et al 1980), it can be calculated for the HOCb:SNP mixture that at 1 min, almost 80% of the injected radioactivity is retained in the plasma. The corresponding figure for the injection of SNP alone is 31%.

It is interesting that injection of SNP in the complexed form did not result in a reduction of hypotensive activity. Presumably the complex acts as a sort of depot in the plasma, releasing the active vasodilator component more slowly than SNP alone. It is unlikely that the 'bulky' complex itself would be able to gain access to the site of action in the smooth muscle to exert any spasmolytic effect in the undegraded form.

Our results using [^{14}C]SNP alone, generally agree with those of Höbel & Raithelhuber (1976) who, using a [^{14}C]SNP dose of 1·3 μmol kg^{-1}, reported that radioactivity in the blood declined rapidly in the first 4–6 min and then fell more slowly. In the present study, because of the relatively low specific activity of the [^{14}C]SNP synthesized, the dose of drug used for the pharmacokinetic studies was about five times higher than the highest dose used in the pharmacodynamic studies. It is therefore difficult to precisely relate plasma concentrations with hypotensive effect, although it could be seen that this effect was associated with a very marked fall in [^{14}C]SNP-derived radioactivity. Presumably the fall in concentration of plasma radioactivity is associated with redistribution of the drug (partly to its site of action in the vascular smooth muscle) as well as its metabolism and excretion.

Mixing ST with SNP in the proportions used (12:1 molar ratio) had no effect on the hypotensive effect of SNP when given as an i.v. bolus dose. This initially appears to disagree with the study of Ivankovich et al (1982) who reported that during a 5 h infusion in dogs, the dose of SNP alone,unlike that of an 18:1 molar ratio ST:SNP mixture, had to be steadily reduced to maintain a constant level of hypotension. However, this necessary dosage reduction with SNP alone was explained on the basis of decreasing cardiac reserve due to the toxicity of accumulating cyanide. No such indirect enhancement of the hypotensive effect of SNP given 'chronically' was detected by Schulz et al (1982) who reported that in a single patient, infusion of an ST:SNP mixture (12:1 molar ratio) was equally or slightly more effective than SNP alone at lowering blood pressure. In the present study, using single bolus doses of SNP or SNP/ST mixture given in a randomized order, any effect of cumulative cyanide cardiotoxicity would not be readily apparent from the meaned data presented. However, examination of the individual hypotensive responses in each rat revealed no increase in efficacy of SNP during the period of each experiment, indicating that cyanide-induced cardiotoxity was not occurring.

The rationale for using a 12:1 molar ratio mixture in the current study and that of Schulz et al (1982) was based on the report (Saunders & Himwich 1950) that the optimum molar cyanide to thiosulphate ratio for rhodanese-mediated detoxication is 2–3, and the fact that each molecule of SNP contains 5 cyanide ligands.

Unlike HOCb, ST did not cause elevated plasma levels of [^{14}C]SNP-derived radioactivity immediately after injection, although the ST addition did result in higher concentrations of radioactivity during the 4–10 min period. Since the pharmacological response to SNP was unaffected, it is presumed that the higher concentrations of radioactivity were due to inactive degradation products, rather than the active species itself. Using a very large dose of SNP (about 20 μmol kg^{-1}) and [^{14}C]SNP of specific activity several hundred times greater than that used in

the present work, Höbel & Raithelhuber (1976) examined some of the SNP degradation products in the blood. The reported proportions of blood SNP, cyanide and thiocyanate at 1, 10 and 40 min, respectively, were approximately 92, 6, 2%; 68, 25, 6%; and 28, 30, 42%.

Compared with ST, the use of HOCb as an adjunct to SNP treatment suffers from a number of disadvantages. Because of the relatively high molecular weight of HOCb, large amounts of it are required. For instance, about 2·5 g of HOCb would be required to 'neutralize' all the cyanide contained within 100 mg of SNP (Cole 1978). Additional disadvantages of HOCb, apart from a greater financial cost and a limited shelf-life, are an apparently greater intrinsic toxicity and lower antidotal efficacy compared with ST (Höbel et al 1980). Yet a further disadvantage, as shown in the present work, is that HOCb and SNP may chemically interact resulting in a prolonged less-predictable pharmacological response.

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