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Sodium Cyanide Antagonism of the Vasodilator Action of Sodium Nitroprusside in the Isolated Rabbit Aortic Strip

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Resistance to sodium nitroprusside (SNP) is uncommon, but its occurrence has led to massive overdoses of SNP and sometimes death. To examine the mechanism responsible for resistance, aortic smooth muscle strips were prepared and dose–response curves for norepinephrine (NE) obtained. SNP alone caused a shift of the dose–response curve for NE to the right. However, this shift was less when the strips were exposed to both SNP and sodium cyanide (CN^-). When CN^- alone was added to the aortic strips, the response to NE was unchanged. In a further group of aortic muscle strips first contracted with NE and then relaxed with SNP, the addition of CN^- caused the muscles to contract again. It is concluded that CN^- antagonizes the action of SNP *in vitro*, and that this antagonism is specific for SNP. (Key words: Anesthetic techniques, hypotension, induced: nitroprusside. Toxicity: cyanide. Sympathetic nervous system, catecholamines: norepinephrine.)

SODIUM NITROPRUSSIDE (SNP) is a direct-acting vasodilator whose value for intraoperative induction of arterial hypotension,[1,2] for decreasing afterload following myocardial infarction,[3] and for the treatment of severe congestive cardiac failure[4] is well established. Resistance and tachyphylaxis in response to SNP have recently been reported, followed in some instances by death.[5–7] The resistance is characterized by a greater than normal requirement for SNP, metabolic acidosis, and an increase in mixed venous oxygen tension. Significantly increased blood cyanide levels have been reported, and Davis *et al.*[8] have suggested that the resistance to SNP is related to an inability to detoxify cyanide. Vesey and Cole[9] believe that hydrogen cyanide is the cause of the metabolic acidosis and the fatalities following the use of SNP. Recently they have shown that plasma cyanide concentrations may increase as much as tenfold after prolonged use of SNP.[10]

The phenomenon of resistance is poorly understood, but the unusually great requirements for SNP seen in cases of tachyphylaxis led us to speculate that free plasma cyanide might itself influence the action of SNP on vascular smooth muscle. This study, using isolated rabbit aortic strips, was designed to investigate such an interaction.

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Methods

Thoracic aortas were obtained from male albino rabbits weighing 1.5–3.0 kg that were killed by a sharp blow to the head. A helical strip of aortic muscle was prepared by the method described by Furchgott and Bhadrokom.[11] Segments of the strips were cut to lengths approximately 20 mm long 4 mm wide, and each was mounted in an organ bath having a working volume of 50 ml. The bath fluid was modified Krebs' solution through which a gas mixture of carbon dioxide, 5 per cent in oxygen, was bubbled continuously.§ The temperature of the organ bath was maintained thermostatically at 37 ± 0.5 C throughout the experiment. Each muscle strip was allowed to equilibrate for at least two hours before being adjusted to a resting tension of 1.0 g. Isometric cumulative dose–response curves for norepinephrine (NE) over the range 10^{-8} to 10^{-4} M were then recorded on a Grass No. 7 polygraph using a Grass force transducer FT036.

The first series of experiments was performed to determine whether sodium cyanide in a concentration of 10^{-4} M caused any change in the normal response of smooth muscle to norepinephrine. Experiments were usually made with two preparations set up side by side. With four muscle preparations a control cumulative dose–response curve for norepinephrine was obtained first, followed after a recovery period of approximately 60 min by a second dose–response curve for norepinephrine in the presence of sodium cyanide 10^{-4} M, added 2 to 5 min earlier. In three more muscle preparations this sequence was reversed, so that the response to norepinephrine in the presence of sodium cyanide was obtained first, followed approximately 60 min later by the control response. This was done to minimize any alteration of muscle sensitivity resulting from previous exposure to high concentrations of norepinephrine.[11] Between dose–response curves, the muscles were allowed to relax to their previous resting tension of 1.0 g, and the organ bath was flushed with fresh buffer every 15 to 20 min.

In the second series of experiments, the effect of sodium cyanide (CN^-) on the action of SNP was studied. The muscle strips were set up in pairs in a

§ Composition of the solution used (millimolar) was: NaCl 11; $NaHCO_3$ 25; KCl 5; NaH_2PO_4 1; $MgCl_2$ 0.5; glucose 11; $CaCl_2$ 1.4.

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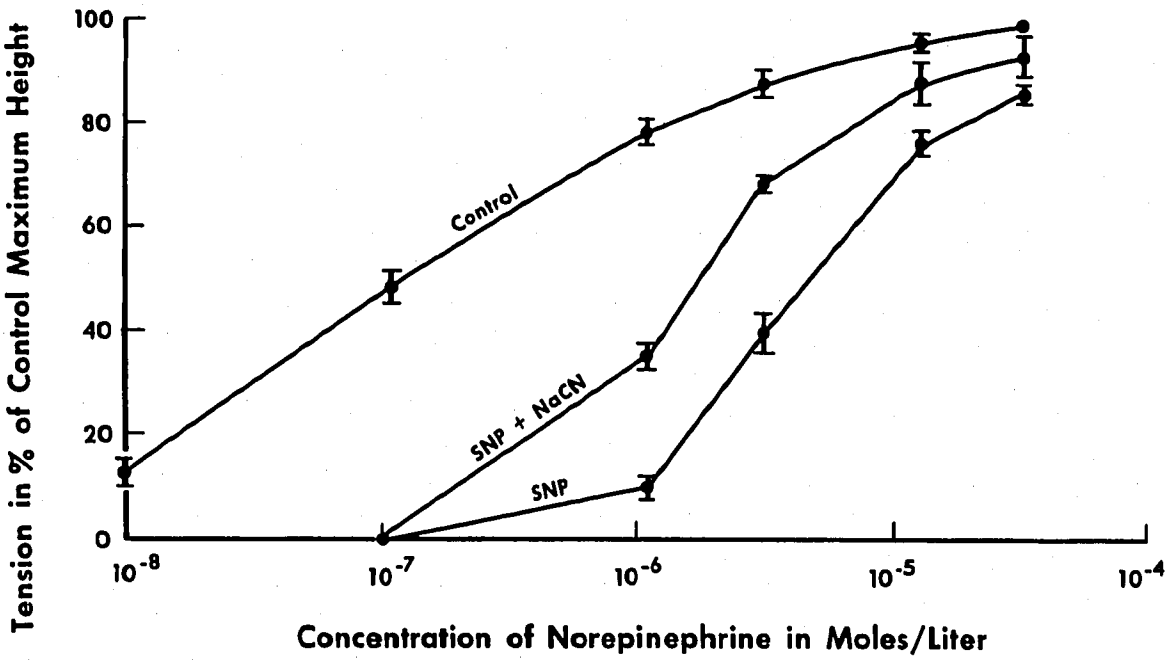

FIG. 1. Dose-response curves obtained for rabbit aortic strips with various concentrations of norepinephrine in mol/l. Tension is expressed as percentage of the control maximum height. SNP (10^{-5} M) was added 2 to 5 min before norepinephrine challenge. SNP (10^{-5} M) and sodium cyanide (10^{-4} M) were added 2 to 5 min before norepinephrine challenge. A significant shift of the dose-response curve to the right occurred with SNP alone, but a significant shift to the left occurred with SNP and sodium cyanide ($P < .05$).

manner similar to that described above except that each strip was used to obtain a sequence of three cumulative dose-response curves for norepinephrine. The first dose-response curve, for norepinephrine alone, was used as the control; the second was obtained in the presence of SNP (10^{-5} M), while the third was obtained in the presence of SNP (10^{-5} M) and sodium cyanide (10^{-4} M), added together 2 to 5 min earlier. Any change in the resting tension of the muscle strip that occurred after the addition of the SNP to the bathing fluid was corrected before the dose-response curve for norepinephrine was obtained. This correction was rarely necessary, and never amounted to more than 5 per cent of the resting tension. Approximately 60 min were allowed for recovery between successive tests, and resting tension was allowed to return to 1.0 g. Eight different muscle strips were used in these experiments. Eight additional muscle strips were challenged with a half-maximal concentration of norepinephrine and the sequence described above was repeated in the reverse manner, *i.e.*, SNP + CN^-, then SNP. The drugs used were: norepinephrine bitartrate, sodium nitroprusside $Na_2Fe(CN)_5NO \cdot 2H_2O$, and sodium cyanide, 99 per cent (J. T. Baker Chemical Co.).

Solutions of drugs were freshly prepared before each experiment using demineralized distilled water and added to the organ bath in 100-μl volumes. Drug concentrations are expressed as mol/l of bath fluid. The data presented are mean values ± standard errors of the mean. Statistical significance of the results was determined using Student's t test for paired data. $P < 0.05$ was considered significant.

Results

In both series of experiments, the addition of norepinephrine alone in concentrations ranging from 1×10^{-8} to 3×10^{-5} M caused dose-related contractions of the muscle preparations. The responses obtained from individual muscle strips are expressed as percentages of the maximum tension produced by norepinephrine alone in the control dose-response curve. The results from each muscle strip have been pooled to construct single dose-response curves for norepinephrine, and each point shown in figure 1 represents the mean of seven or eight values together with the standard error of the mean.

Sodium nitroprusside (10^{-5} M) alone caused significant decreases in tension at all concentrations of norepinephrine, compared with the control response (fig. 1). The addition of sodium cyanide (10^{-4} M) clearly enhanced the response to norepinephrine in the presence of SNP, and there was a shift of the dose-response curve to the left. The response to norepinephrine in the presence of both drugs was not

significantly different from the control response at concentrations of 10^{-5} M or more.

Table 1 illustrates the dose ratios for norepinephrine necessary to product 25, 50, and 75 per cent of the maximum control response in the presence of SNP alone, and SNP with sodium cyanide. A comparison of the two dose ratios for each muscle strip indicates that SNP is approximately three times more effective at inhibiting contractions induced by norepinephrine when sodium cyanide is not present.

It was found that the addition of sodium cyanide to the muscle strips in a concentration of 10^{-4} M caused no significant change in the response to norepinephrine (n = 7). There was no indication that exposure of the muscle strip to sodium cyanide in the first dose–response curve experiment produced any toxic effect that might have altered the response in the second. However, in earlier experiments using sodium cyanide at concentrations of 3×10^{-4} M or more, we did observe some reduction of the contractile response of the muscle strips to norepinephrine, particularly when approaching maximum tension. Any alteration in response of the preparations to norepinephrine and SNP caused by the presence of sodium cyanide at a concentration of 10^{-4} M was thus unlikely to be the result of any histotoxic effect on the muscle itself.

While it is known that SNP produces its vasodilatory effect *in vivo* for only a few minutes, it was found that its relaxation of the vascular smooth muscle preparations described here lasted substantially longer. Muscle strips contracted by norepinephrine could be made to relax for at least 60 min by the addition of SNP (10^{-5} M) to the bathing fluid. Since all dose–response curves obtained in the subsequent experiments were completed less than 30 min after the addition of SNP, its duration of action was considered adequate for the purpose of this study.

It was observed, however, that after maximal contraction of the muscle strips with 2×10^{-5} M norepinephrine, the relaxation produced by SNP (10^{-5} M) was not completely maintained, and there was some recovery of tension after 30 min (equivalent to approximately 8 per cent of the maximum relaxation induced by SNP) (fig. 2). No recovery of tension was seen when lower concentrations of norepinephrine were used.

In the second series of experiment, the sequence of dose–response curves was altered so that the control responses to norepinephrine were always obtained first. Following initial exposure of a muscle strip to high concentrations of norepinephrine, sensitivities in subsequent exposures were always decreased to less than that in the first exposure. These decreases were greatest at contraction levels less than half maximal; at higher levels of contraction the difference in sensitivity became less marked. However, this decreased sensitivity recovered with time and was complete within 2 to 3 hr.[11] Since in the experiments described here the intervals between the first and second exposures to norepinephrine were approximately similar to those between the second and third exposures, the sensitivities in the latter two dose–response curves would normally be similar. The additional experiments performed using half maximal contraction did not alter the findings when SNP and CN preceded SNP alone.

TABLE 1. Dose Ratios of Norepinephrine Needed to Produce 25, 50 and 75 per cent of Control Response in the Presence of Sodium Nitroprusside (10^{-5} M) and Sodium Nitroprusside (10^{-5} M) with Sodium Cyanide (10^{-4} M) (Values are Means ± SEM)

	Dose Ratio with SNP	Dose Ratio with SNP + CN	Dose Ratio with SNP / Dose Ratio with SNP + CN	Number of Strips
ED_{25}	73.9 ± 5.4	25.75 ± 3.3*	3.13 ± .29	7
ED_{50}	56.6 ± 16.2	17.37 ± 3.8*	2.98 ± .27	8
ED_{75}	40.8 ± 14.7	12.75 ± 5.4*	3.40 ± 1.20	8

* Significantly different from sodium nitroprusside alone, t test for paired data, $P < .05$.

In another series of experiments, rabbit aortic strips prepared as described above were contracted by norepinephrine (10^{-6} M) and then SNP (10^{-5} M) was added, causing the muscles to relax. When the tension was stable, the further addition of sodium cyanide to the organ bath produced an immediate increase in tension (fig. 3). Lower concentrations of SNP (10^{-7} M) and CN (10^{-6} M) produced similar results. This confirmed the findings of the previous experiments and also demonstrated the rapidity with which cyanide appears to antagonize the action of SNP. The specificity of this response is indicated by the failure of sodium cyanide (10^{-4} M) to affect relaxation of smooth muscle strips by a variety of directly-acting smooth-muscle relaxants. These were adenosine (5×10^{-5} M), glyceryl trinitrate (10^{-6} M) and papaverine (1.8×10^{-5} M). The doses were selected to produce relaxation comparable to that obtained with SNP 10^{-5} M.

Discussion

It is now generally accepted that cyanide is a product of the breakdown of SNP. Smith and Kruszyna[12] have demonstrated that the likely mechanism for the breakdown of SNP is a rapid, non-enzymatic process involving free and intracellular hemoglobin. In this

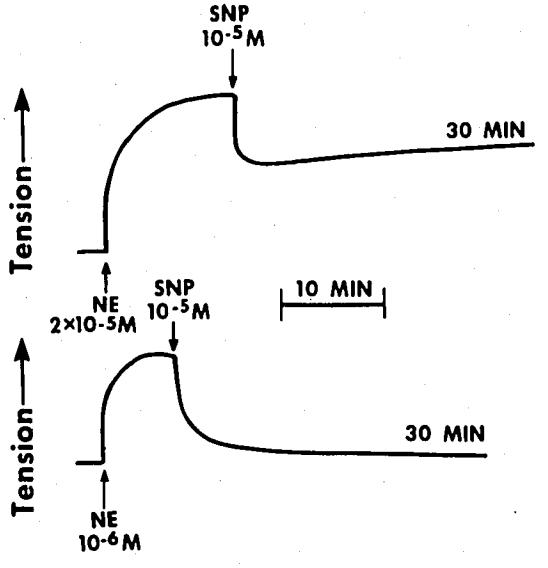

FIG. 2. Plot illustrating an example of the relaxation produced by SNP in norepinephrine-contracted muscle preparations. Recovery of tension 30 min after introduction of SNP in maximally contracted muscles was equivalent to 8 per cent of the relaxation produced (n = 9).

process each molecule of SNP releases five cyanide ions, one of which combines with methemoglobin to form cyanmethemoglobin while the other four are free to exert their characteristic effects. A portion of the free cyanide is then converted to thiocyanate in the liver and kidneys by the enzyme rhodanase. Rhodanase appears to require adequate amounts of endogenous thiosulfate to effect this conversion.

In the past, the production of free cyanide from the metabolism of SNP was thought to be minimal. However, Vesey *et al.*[10] have shown that there is a significant increase in plasma cyanide concentrations following the infusion of SNP. They demonstrated that even after short periods of infusion, there is a significant correlation between the increase in plasma cyanide concentration and both the total dose and the mean rate of SNP infusion. They reported that where post-infusion cyanide concentrations were high, the subsequent decreases in both erythrocytic and plasma concentrations were much slower. This appears to indicate the likelihood of population variability in the rate of cyanide detoxification, so that in some patients excessive accumulation might occur.

Davies *et al.*[8] have successfully reversed tachyphylaxis to SNP. The abnormally great SNP requirements, increased blood cyanide levels, and signs of tissue hypoxia could all be reversed by administering sodium thiosulfate intravenously. They propose that the apparent disturbance in the cyanide-to-thiocyanate pathway results from either an inadequate supply of endogenous thiosulfate, a deficiency or an abnormality of tissue rhodanase, an inhibition of tissue rhodanase by other drugs, or a combination of these. An increase in plasma cyanide concentration may give rise to metabolic acidosis as a result of tissue hypoxia. This would help to explain some decrease in rhodanase activity, since the action of this enzyme is *p*H-sensitive. Saunders and Himwich[13] have shown that rhodanase has an optimum *p*H of 9.1 and that a decrease in intracellular *p*H from 7.2 to 6.9 can decrease its activity by as much as 40 per cent.

Since the breakdown of SNP is non-enzymatic, the unusually large amount needed to maintain a constant hypotensive effect in a patient manifesting tachyphylaxis does not seem to be explained by a disturbance in the cyanide-to-thiocyanate pathway, unless it is the result of accumulation of the intermediate products of SNP breakdown. The results of this study, indicating that cyanide can significantly decrease the action of SNP in isolated smooth muscle, appear to confirm the possibility that an increased serum cyanide concentration may be responsible, at least in part, for the decreased sensitivity to SNP when tachyphylaxis is observed.

There are three ways in which cyanide ions might

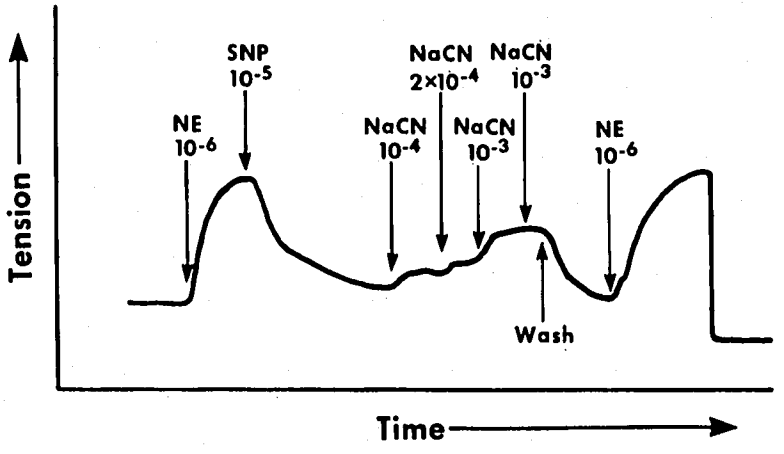

FIG. 3. Plot illustrating an example of responses of an aortic strip contracted with norepinephrine and relaxed with SNP, and the subsequent response to sodium cyanide. After washing of the aortic strip, a norepinephrine challenge produced a response similar to the initial contraction.

antagonize the action of SNP: 1) a direct toxic effect on the mechanisms responsible for smooth-muscle relaxation; 2) chemical antagonism, perhaps by the formation of ferrocyanide; 3) pharmacologic antagonism of SNP at its "receptor" site.

Needleman[14] has proposed a model for the mechanism of action of directly-acting vasodilators in which SNP has a specific receptor site but acts through a common intermediate reaction involving critical sulfhydryl groups within the tissue. The presence of cyanide at concentrations of less than 10^{-4} M caused no alteration in the contractile response to norepinephrine, so it seems unlikely that it would affect the relaxation mechanism directly. In addition, cyanide did not alter the relaxation of smooth muscle strips caused by the presence of glyceryl trinitrate, adenosine, or papaverine, all of which are thought to act through the common intermediary reaction involving the critical sulfhydryl groups. Metabolic inhibition of oxidative metabolism in smooth muscle by cyanide might be more likely to contribute to the relaxation induced by SNP, rather than the reverse.

Chemical antagonism of SNP by cyanide might also be considered, since replacement of the NO^+ group on the nitroprusside ion with cyanide would produce ferrocyanide. Photodecomposition of SNP in alkaline or acidic conditions and in the presence of cyanide might in time produce a reaction, but at a relatively slow rate. In an experiment where SNP and cyanide were mixed together in stoppered tubes and exposed to diffused light for 15 min, there was no difference between the hydrogen cyanide yields of SNP with cyanide and cyanide alone (Vesey CJ: Personal communication). It is, therefore, very unlikely that any chemical interaction is responsible for the antagonism by cyanide of the action of SNP.

The shift of the dose–response curves to the left indicates the possibility of pharmacologic antagonism (fig. 1). The specificity of cyanide in antagonizing only the action of SNP and not those of the other directly-acting vasodilators suggests that there may be reversible interference with the formation of the drug receptor complex. The kinetics of such a competitive inhibition would be dependent on the ratio of drug concentrations. The antagonism was increased by increasing the cyanide concentration to 2×10^{-4} M (fig. 3), and it could also be decreased by increasing the SNP concentration, indicating that this is a likely mechanism of action.

This *in-vitro* study suggests that the resistance to SNP seen in some patients may be related to an increasing serum cyanide level, which causes an apparent ineffectiveness of SNP. Since the antagonism was seen only when the cyanide concentration exceeded the SNP concentration, immediate resistance to SNP would not necessarily be seen. However, once the cyanide level approached the SNP concentration, resistance would be expected to become evident. More SNP is then administered to decrease the blood pressure, and more cyanide is produced, which further diminishes the effectiveness of SNP as a vasodilator. As with many positive-feedback loops, this situation would lead to an unstable and potentially dangerous vicious circle.

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