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The effects of sodium nitroprusside and 8-bromo-cyclic GMP on electrical and mechanical activities of the rat tail artery

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1 The effects of sodium nitroprusside and 8-bromo-guanosine 3':5'-cyclic monophosphate (8-bromo-cyclic GMP) on the electrical and mechanical activities of the rat tail artery were compared.

2 The inhibitory effects of sodium nitroprusside on the contractions induced by noradrenaline, phenylephrine, KCl and clonidine were mimicked by 8-bromo-cyclic GMP.

3 Sodium nitroprusside and 8-bromo-cyclic GMP increased the resting membrane potential only in preparations with low initial resting membrane potentials.

4 In tissues previously contracted and depolarized with noradrenaline, KCl and clonidine, both sodium nitroprusside and 8-bromo-cyclic GMP caused relaxation without significantly affecting the membrane potential.

5 Both sodium nitroprusside and 8-bromo-cyclic GMP abolished neurally-mediated contractions without any significant effect on the electrical responses.

6 These results suggest that the actions of sodium nitroprusside and 8-bromo-cyclic GMP are not related to membrane hyperpolarization or inhibition of membrane excitability.

Introduction

Sodium nitroprusside is a hypotensive drug that causes dilatation of both resistance and capacitance vessels. It has a direct effect on vascular smooth muscle, since sympathectomy, adrenoceptor blocking drugs and cholinoceptor blocking drugs fail to alter its effects (Johnson, 1929; Page *et al.*, 1955; Kreye *et al.*, 1975; Verhaeghe & Shepherd, 1976). Recently, two hypotheses concerning the mechanism of action of sodium nitroprusside have gained considerable interest. One implicates hyperpolarization of the smooth muscle membrane in sodium nitroprusside-induced relaxation, which would shift the membrane potential away from the threshold required for contraction (Haeusler & Thorens, 1976). The work of other investigators offers a different approach towards understanding of the mechanism of action of sodium nitroprusside. Since significant increases in guanosine 3':5'-cyclic monophosphate (cyclic GMP) have been shown to precede sodium nitroprusside-induced relaxation in various smooth muscle preparations, it has been suggested that cyclic GMP mediates the relaxation (Katsuki *et al.*, 1977; Schultz *et al.*, 1977). Interestingly, Edwards *et al.* (1984) noted a difference in the ability of sodium nitroprusside to relax contracted arteries and veins, which corresponded to its different ability to generate cyclic GMP in the two preparations.

In order to test these hypotheses, we employed a technique which allows simultaneous recording of both electrical and mechanical activities of blood vessels. To conclude that hyperpolarization underlies relaxation, obviously a causal relationship must exist. Simultaneous recording of membrane potential and tension could establish this relationship, or lack thereof. If increased cyclic GMP formation underlies relaxation, then cyclic GMP would be expected to mimic the effects of sodium nitorprusside. In the present study, we compared the effects of sodium nitroprusside and 8-bromo-cyclic GMP on the electrical and mechanical activities of the rat tail artery.

Methods

The experimental procedure used was similar to that previously described (Cheung, 1984). Tail arteries were dissected from male Wistar rats weighing 250–350 g. Ring segments 3–4 mm in length were

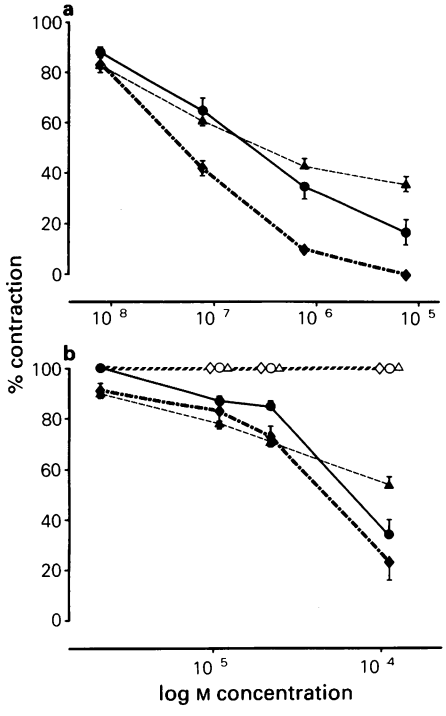

Figure 1 Effects of sodium nitroprusside (a), cyclic GMP (b, open symbols) and 8-bromo-cyclic GMP (b, closed symbols) on contractions induced by (▲) 90 mM KCl; (●) 3.1×10^{-6}M noradrenaline; (◆) 4.3×10^{-6}M clonidine in the rat tail artery. Sodium nitroprusside caused dose-dependent relaxation, while cyclic GMP had no effect. The lipophilic analogue of cyclic GMP, 8-bromo-cyclic GMP, caused significant relaxation at the highest dose used. Each point represents the mean of at least 5 experiments, s.e.mean shown by vertical bars.

mounted in an organ bath and superfused with oxygenated physiological salt solution (composition, mM: NaCl 120, $NaHCO_3$ 25, glucose 11, KCl 5, $CaCl_2.2H_2O$ 2.5, $NaH_2PO_4.H_2O$ 1 and $MgSO_4.7H_2O$ 1) at 36–37°C. Propranolol (1×10^{-5}M) was added to the solution for all experiments, and for those involving the use of KCl, phentolamine-(3.6×10^{-7}M) was also added. To measure tension, two fine tungsten wires were inserted through the lumen of the artery. One wire served as an anchor and the other wire was connected to a Narco F-60 force transducer mounted on a micromanipulator to allow tension adjustments. An initial tension of 100 mg was applied to the artery.

For stimulation of the perivascular nerves, the anchor wire and a third wire running parallel to the tissue were used as stimulating electrodes. Stimuli were generated from a Grass S48 stimulator and were of 0.1 ms in duration. For intracellular recordings, fibre-filled glass micropipettes filled with 3 M KCl and of 30–50 MΩ resistance were used. Before recordings were made, the tissue was allowed at least 1 h to equilibrate. Recordings were displayed on a Gould OS 1420 oscilloscope and stored on a TEAC cassette recorder. Student's *t* tests and analysis of variance were used for statistical analysis of data, and a *P* value of less than 0.05 was considered statistically significant.

The following drugs were used: propranolol, noradrenaline, sodium nitroprusside and 8-bromo-cyclic GMP (Sigma), phentolamine (Ciba), clonidine (Boehringer Ingelheim).

Results

Mechanical effects of sodium nitroprusside and 8-bromo-cyclic GMP in the rat tail artery

In the rat tail artery, contractions induced by 3.1×10^{-6}M noradrenaline, 4.3×10^{-6}M clonidine and 90 mM KCl were found to be relaxed by both sodium nitroprusside and 8-bromo-cyclic GMP. The relaxant effects of sodium nitroprusside were dose-dependent over the range of 7.6×10^{-9}M to 7.6×10^{-6}M (Figure 1a). In contrast, only high concentrations of 8-bromo-cyclic GMP were effective in causing relaxation (Figure 1b). Relaxation was not observed with cyclic GMP, even at high doses of the cyclic nucleotide (Figure 1b). Therefore, only 8-bromo-cyclic GMP was used in subsequent studies.

The contractions induced by noradrenaline and phenylephrine were inhibited by sodium nitroprusside (7.6×10^{-7}M) only at lower dose ranges of the agonists and had no effect when maximally stimulated. However, the KCl-induced contraction was suppressed throughout the entire dose-range, up to 150 mM. Maximum tension development in response to clonidine was only 45% of that to noradrenaline and sodium nitroprusside was most effective in abolishing the clonidine-induced contraction. Even at high doses of clonidine, inhibition was almost complete. Thus, the effect of sodium nitroprusside was different for different agonists. In all cases, the effect of sodium nitroprusside could be mimicked by 8-bromo-cyclic GMP (1.1×10^{-4}M) (Figure 2).

Effects of sodium nitroprusside and 8-bromo-cyclic GMP on resting membrane potential

The effect of sodium nitroprusside and 8-bromo-cyclic GMP on the resting membrane potential was related to the initial control resting membrane potential of the tissue. Hyperpolarization in response to these agents was larger in tissues with lower control membrane

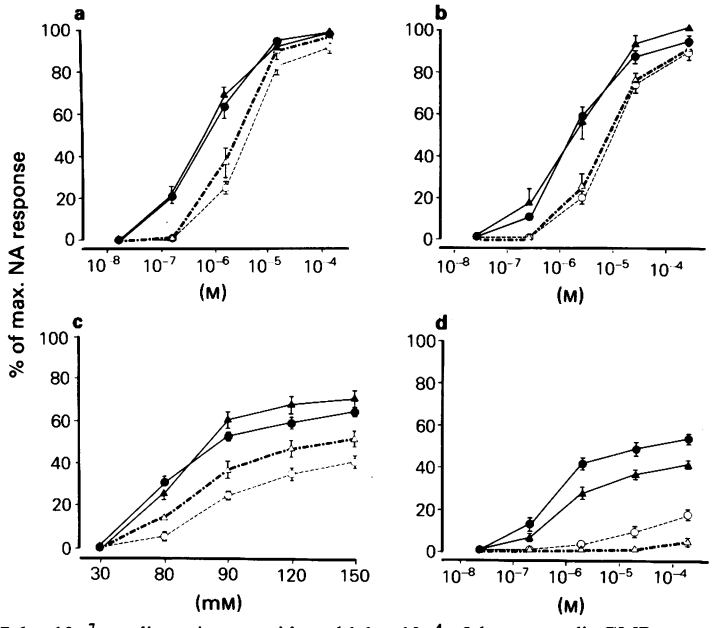

Figure 2 Effects of 7.6×10^{-7}M sodium nitroprusside and 1.1×10^{-4}M 8-bromo-cyclic GMP on contractile responses to (a) noradrenaline, (b) phenylephrine, (c) KCl, and (d) clonidine in the rat tail artery. (●) Control for sodium nitroprusside; (○) sodium nitroprusside; (▲) control for 8-bromo-cyclic GMP; (△) 8-bromo-cyclic GMP. Sodium nitroprusside and 8-bromo-cyclic GMP had a greater effect at lower dose ranges of noradrenaline and phenylephrine. The responses to KCl were similarly inhibited throughout the entire dose-range. The responses to clonidine were most effectively suppressed by both sodium nitroprusside and 8-bromo-cyclic GMP. Each point represents the mean of at least 5 experiments, s.e.mean shown by vertical bars. The molar concentrations for noradrenaline, phenylephrine and clonidine are given on a log scale.

potential. The maximum membrane potential reached in all cases did not exceed −72 mV (Table 1). Both sodium nitroprusside and 8-bromo-cyclic GMP had no significant effect on resting tension.

Relationship between tension and membrane potential

The effects of sodium nitroprusside (7.6×10^{-7}M) and 8-bromo-cyclic GMP (1.1×10^{-4}M) on tissues

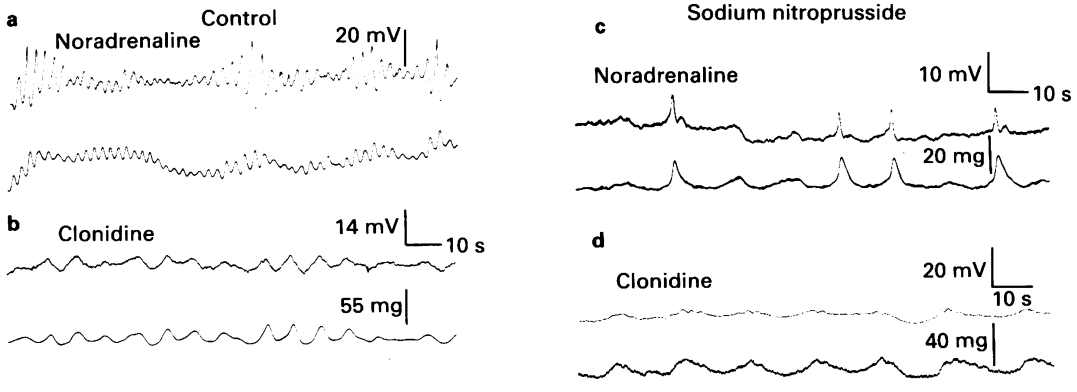

Figure 3 Coupled oscillation of membrane potential (top traces) and tension (bottom traces) was occasionally observed in rat tail arteries treated with noradrenaline (a) and clonidine (b). Sodium nitroprusside (7.6×10^{-7}M) did not suppress the coupled oscillations produced by noradrenaline (c) and clonidine (d). Note that the sustained tension had decreased by 72% (c) and 71% (d) with the addition of sodium nitroprusside.

Table 1 The effect of sodium nitroprusside and 8-bromo-cyclic GMP on the resting membrane potential of the rat tail artery

Effect of sodium nitroprusside on membrane potential (mV):					
Control	56.0 ± 0.8*	66.0 ± 0.4*	66.8 ± 1.0	67.3 ± 0.6	65.1 ± 1.1*
Sodium nitroprusside (3.8×10^{-8}M)	72.2 ± 1.0	68.5 ± 0.8	66.0 ± 1.1	69.0 ± 1.2	69.3 ± 0.5
Control	56.0 ± 0.8*	66.0 ± 0.4*	66.8 ± 1.0	67.3 ± 0.6	65.1 ± 1.1*
Sodium nitroprusside (3.8×10^{-7}M)	69.8 ± 0.2	71.8 ± 0.9	71.0 ± 2.4	68.8 ± 0.6	69.2 ± 0.6
Control	72.8 ± 2.2	70.2 ± 1.8	67.7 ± 3.3	66.4 ± 1.4*	67.8 ± 1.9*
Sodium nitroprusside (7.6×10^{-7}M)	72.0 ± 2.8	72.2 ± 3.6	69.2 ± 3.3	69.2 ± 0.8	70.8 ± 1.3
Effect of 8-bromo-cyclic GMP on membrane potential (mV):					
Control	67.2 ± 0.3	66.0 ± 0.5	68.2 ± 1.0	67.0 ± 0.7*	69.2 ± 0.6
8-bromo-cyclic GMP (1.1×10^{-5}M)	67.0 ± 1.6	66.5 ± 0.7	67.2 ± 0.9	70.4 ± 0.6	68.0 ± 0.6
Control	66.0 ± 0.5	67.0 ± 0.7*	69.2 ± 0.6	68.2 ± 1.0	
8-bromo-cyclic GMP (1.1×10^{-4}M)	66.0 ± 0.6	70.3 ± 0.6	70.7 ± 0.4	66.7 ± 0.6	
Control	58.6 ± 1.3	52.2 ± 1.4*	67.0 ± 0.7		
8-bromo-cyclic GMP (2.2×10^{-4}M)	67.6 ± 1.9	63.2 ± 1.8	66.4 ± 0.8		

Membrane potentials (mV) are expressed as mean ± s.e.mean ($n = 5$ cells) of each preparation before and after incubation in either sodium nitroprusside or 8-bromo-cyclic GMP for at least 45 min. * indicates significant change in membrane potential ($P < 0.005$). Analysis of variance indicates that there is a significant correlation ($P < 0.001$) between the initial control membrane potential and the hyperpolarization response to sodium nitroprusside ($F_{1,13} = 87.12$) and 8-bromo-cyclic GMP ($F_{1,10} = 47.39$).

previously treated with various agonists are shown in Table 2. Noradrenaline (3.1×10^{-6}M) produced contraction and depolarized the membrane to −38 mV. In 2 out of 11 preparations, noradrenaline produced oscillation of both the contractile and electrical responses. These oscillations were electro-mechanically coupled in that changes in membrane potential preceded changes in tensions (Figure 3a). Clonidine

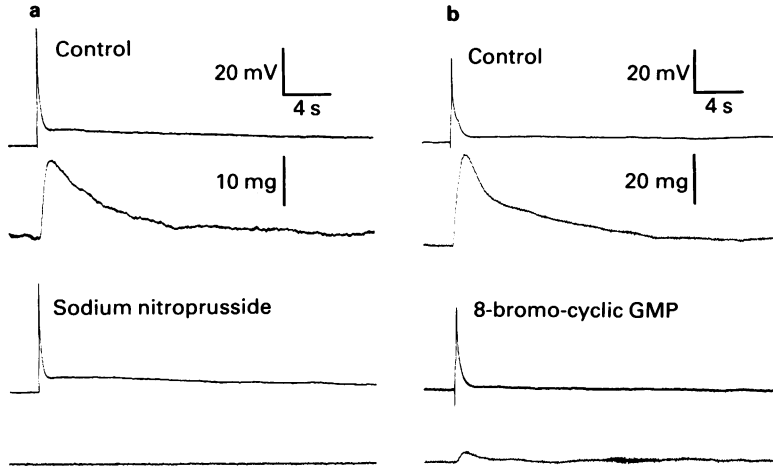

Figure 4 Effects of 3.8×10^{-7}M sodium nitroprusside (a) and 2.2×10^{-4}M 8-bromo-cyclic GMP (b) on electrical and mechanical responses to nerve stimulation in the rat tail artery. Pulses were of 100 V and 0.1 ms duration. Both relaxants suppressed contractile responses without significant effects on membrane excitability.

Table 2 Effect of 8-bromo-cyclic GMP as compared to sodium nitroprusside on membrane depolarization and contraction induced by noradrenaline, KCl and clonidine

Sodium nitroprusside			*8-bromo-cyclic GMP*		
Noradrenaline	*Membrane potential (mV)*	*% relaxation*	*Noradrenaline*	*Membrane potential (mV)*	*% relaxation*
Control	67.6 ± 3.0		Control	67.7 ± 2.2	
NA (3.6×10^{-6}M)	38.5 ± 0.9	71.0 ± 15.4	NA (3.6×10^{-6}M)	38.2 ± 1.2	66.0 ± 6.6
Sodium nitroprusside (7.6×10^{-7}M)	41.6 ± 2.5		8-bromo-cyclic GMP (1.1×10^{-4}M)	37.9 ± 0.5	
KCl			*KCl*		
Control	72.5 ± 1.2		Control	71.1 ± 1.2	
KCl (90 mM)	15.0 ± 0.7	32.4 ± 4.0	KCl (90 mM)	17.8 ± 1.6	44.2 ± 9.3
Sodium nitroprusside (7.6×10^{-7}M)	14.6 ± 1.0		8-bromo-cyclic GMP (1.1×10^{-4}M)	17.7 ± 1.4	
Clonidine			Clonidine		
Control	67.8 ± 1.1		Control	66.7 ± 1.6	
Clonidine (4.3×10^{-6}M)	33.8 ± 2.1	76.0 ± 7.1	Clonidine (4.3 ± 10^{-6}M)	34.2 ± 2.2	80.0 ± 5.9
Sodium nitroprusside (7.6×10^{-7}M)	41.8 ± 3.2		8-bromo-cyclic GMP (1.1×10^{-4}M)	39.1 ± 2.4	

Membrane potentials (mV) are expressed as mean ± s.e.mean ($n = 5$ experiments with 5 cells each). In tissues showing oscillations in the presence of noradrenaline and clonidine, membrane potential and tension measurements were taken during the quiescent periods.
% relaxation is the change in tension after incubation in either sodium nitroprusside or 8-bromo-cyclic GMP for at least 45 minutes.

(4.3×10^{-6}M) produced contraction and depolarized the membrane to about −34 mV, and coupled oscillatory activity was observed in 7 out of 10 preparations (Figure 3b). For both agonists, the frequency and amplitude of the oscillations varied with tissue and time, with intermittent quiescent periods. KCl (90 mM) produced contraction and depolarized the membrane to about −17 mV; however, oscillatory activity was never observed.

Incubation with sodium nitroprusside reduced developed tension in response to noradrenaline, clonidine and KCl. However, the coupled oscillatory activity in response to noradrenaline and clonidine continued in the presence of the relaxant (Figure 3c,d). No significant change in membrane potential accompanied this relaxant effect (Table 2). Incubation with 8-bromo-cyclic GMP also reduced developed tension in response to noradrenaline, clonidine and KCl (Table 2). Again, no significant changes in membrane potential could be observed with relaxation.

In these studies, recordings of electrical and mechanical activities occurred over a period of about 1 h. During this time, clonidine and KCl contractions were relatively stable, with a spontaneous decay of the tension by less than 10%. However, noradrenaline contractions generally decayed by 10–20%.

Effect of sodium nitroprusside and 8-bromo-cyclic GMP on responses to nerve stimulation

In the rat tail artery, stimulation of perivascular nerves with single pulses of sufficient intensity elicits an action potential associated with contraction (Cheung, 1984). At a concentration of 3.8×10^{-7}M, sodium nitroprusside greatly reduced or abolished neurally-mediated contraction. However, the action potential was not significantly affected by the relaxant (Figure 4a). At a concentration of 2.2×10^{-4}M 8-bromo-cylic GMP also reduced or abolished contraction without significantly affecting the action potential (Figure 4b). Thus both agents inhibited contraction without affecting membrane excitability.

Discussion

The proposal that sodium nitroprusside causes relaxation by shifting the membrane potential away from the required threshold for contraction (Haeusler & Thorens, 1976) was directly tested in the present study by simultaneously measuring membrane potential and tension in the rat tail artery. Our results were not consistent with the hypothesis that relaxation is direct-

ly mediated by membrane hyperpolarization. Tissues contracted and depolarized by noradrenaline, clonidine and KCl were all relaxed by sodium nitroprusside without significant change in membrane potential.

The results of this study suggest that sodium nitroprusside may increase the resting membrane potential by increasing K^+ permeability. In tissues with low resting membrane potentials, sodium nitroprusside increased the membrane potential to about -70 mV. However, in tissues with resting membrane potentials initially close to this value, sodium nitroprusside produced little or no increase in membrane potential. That a membrane potential of about -70 mV was consistently observed in the presence of sodium nitroprusside, regardless of the initial resting membrane potential, suggested that sodium nitroprusside increased K^+ permeability. The final value of -70 mV may reflect the equilibrium potential for K^+. That sodium nitroprusside increases K^+ permeability is consistent with reports that sodium nitroprusside decreases membrane resistance in other arteries (Ito *et al.*, 1978; Itoh *et al.*, 1981).

Based on an observed sensitivity to ouabain, Haeusler & Thorens (1976) attributed sodium nitroprusside-induced hyperpolarization in the rabbit main pulmonary artery to an increase in activity of the electrogenic Na^+/K^+ pump. However, recent evidence suggests that ouabain also decreases membrane permeability to K^+ in addition to its effects on the electrogenic pump (Hirst & Van Helden, 1982). Consequently, conclusions that hyperpolarization is due to an increase in electrogenic pump activity based on an observed sensitivity to ouabain may not be entirely valid.

Recent studies have suggested that the major site of action of sodium nitroprusside is intracellular rather than at the level of the membrane. In particular, it has been proposed that sodium nitroprusside inhibits intracellular Ca^{2+} release since, (1) contractions produced independently of external Ca^{2+} were susceptible to relaxation by sodium nitroprusside (Heaslip & Rahwan, 1983; Karaki *et al.*, 1984) and (2) sodium nitroprusside reduced the amount of $^{45}Ca^{2+}$ released from canine renal arteries into external Ca^{2+}-free plus EDTA solution (Hester & Weiss, 1984). Our study supports the possibility that sodium nitroprusside inhibits intracellular Ca^{2+} release. In the rat tail artery, a large fraction of the contraction induced by noradrenaline is not mediated by membrane potential change since, (1) contractions were not correlated with membrane depolarization (Cheung, 1984), (2) large contractions may be induced when the membrane potential is still below threshold for electro-mechanical coupling (Cheung, 1984) and (3) contractions were not very sensitive to Ca^{2+} agonists or Ca^{2+}-entry blockers (unpublished observations). If noradrenaline causes contraction by a release of internal Ca^{2+} (Heaslip & Rahwan 1983; Karaki *et al.*, 1984), then sodium nitroprusside would be expected to produce relaxation.

The effects of sodium nitroprusside on neural responses were consistent with a mechanism whereby sodium nitroprusside inhibits internal Ca^{2+} release. Contractions associated with action potentials in arteries are due to a Ca^{2+}-induced Ca^{2+} release, whereby a small amount of Ca^{2+} entering the cell during the action potential triggers a massive release of Ca^{2+} from internal stores (Itoh *et al.*, 1981; 1982). If a similar mechanism exists in the rat tail artery then sodium nitroprusside could inhibit intracellular Ca^{2+} release and therefore contraction without affecting the action potential, as was observed in the present study. The lack of effect on the action potential also indicates that sodium nitroprusside did not interfere with the influx of external Ca^{2+} through the calcium channels.

KCl-induced contractions in the rat tail artery have been shown to be mediated by electro-mechanical coupling since, (1) membrane depolarization precedes contraction (Cheung, 1984), (2) a threshold level of depolarization is required for the initiation of contraction (Cheung, 1984), and (3) contractions are very sensitive to Ca^{2+} agonists and Ca^{2+}-entry blockers (unpublished observations). Contractions induced by KCl in the tail artery were also partially sensitive to the relaxant effect of sodium nitroprusside suggesting that intracellular Ca^{2+} may contribute to the contraction. In the dog coronary artery, contractions induced by KCl are due to an internal release of Ca^{2+} triggered by Ca^{2+} entering through potential-operating channels (Imai *et al.*, 1984). In the guinea-pig portal vein, high potassium can cause contraction in Ca^{2+}-free solution by inducing release of internal Ca^{2+} from the sarcoplasmic reticulum (Bond *et al.*, 1984). If a similar mechanism is operative in the rat tail artery, then a sensitivity to sodium nitroprusside would be expected.

Contractions induced by clonidine were most susceptible to sodium nitroprusside. It is possible that in addition to inhibition of internal Ca^{2+} release, other factors such as accelerated Ca^{2+} extrusion (Suematsu *et al.*, 1984) may contribute significantly to its inhibitory effect.

Activation of tissues with noradrenaline and clonidine produced electro-mechanically coupled oscillatory activity which was not affected by sodium nitroprusside. This would be expected if changes in membrane potential regulate external Ca^{2+} influx through potential-operated channels. That this is likely to be the case is supported by the finding that changes in membrane potential immediately preceded changes in tension. Presumably, the sodium nitroprusside-induced reduction in developed tone was due to inhibition of contraction mediated by internal Ca^{2+} release. These conclusions are consistent with those of

Hester & Weiss (1984), who found that in the canine renal artery and vein, noradrenaline produced both a sodium nitroprusside-sensitive increase in basal tone and sodium nitroprusside-resistant oscillation. The oscillations were found to be inhibited by Ca^{2+} entry blockers.

A number of studies have shown that sodium nitroprusside significantly increases cyclic GMP levels in vascular smooth muscle (Katsuki *et al.*, 1977; Schultz *et al.*, 1977; Keith *et al.*, 1982; Edwards *et al.*, 1984). Furthermore, it has been demonstrated that the lipophilic analogue of cyclic GMP, 8-bromo-cyclic GMP, causes relaxation in the rabbit aorta (Schultz *et al.*, 1977). Methylene blue antagonized both relaxation and increased cyclic GMP formation induced by sodium nitroprusside (Keith *et al.*, 1982; Edwards *et al.*, 1984). Our results suggest that cyclic GMP may mediate sodium nitroprusside-induced relaxation based on the following findings: (1) Contractions induced by various agonists showed differences in susceptibility to sodium nitroprusside. These differences in susceptibility were mimicked by 8-bromo-cyclic GMP. (2) Both sodium nitroprusside and 8-bromo-cyclic GMP increased the membrane potential of the tail artery to about −70 mV, but only in tissues with a low resting membrane potential. (3) In tissues contracted and depolarized by various agonists, 8-bromo-cyclic GMP mimicked the ability of sodium nitroprusside to cause relaxation without affecting the membrane potential. (4) Both sodium nitroprusside and 8-bromo-cyclic GMP inhibited neurally-mediated contraction without suppressing membrane electrical activities.

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