Sodium Nitroprusside Enhances Contractions of the Guinea-pig Isolated Vas Deferens

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Abstract

The effects of sodium nitroprusside on the electrical and mechanical properties of the smooth muscle of the guinea-pig vas deferens, and its responses to transmitter substances, have been investigated by use of the sucrose-gap technique.

Isolated longitudinal segments of guinea-pig vas deferens contracted in response to electrical field stimulation (100 V, 0.04–0.1 ms, 1–5 Hz, 10 s train every 60 s) and application of ATP (1 mM) or noradrenaline (10 μM). Sodium nitroprusside (0.1 mM) did not affect resting tension but did enhance contractions evoked by electric-field stimulation but not by ATP or noradrenaline. The sodium nitroprusside-induced enhancement was unaffected by the nitric oxide synthase inhibitor, N^ω-nitro-L-arginine methyl ester (L-NAME) (0.1 mM). Conversely, electrically evoked contractions were unaffected by the nitric oxide precursor L-arginine (1 mM) or the nitric oxide donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) (0.1 mM). The amplitudes of electrically evoked excitatory junction potentials (EJPs) were not affected by application of sodium nitroprusside, although it caused a small depolarization of 0.7 ± 0.3 mV. Similarly, the depolarization caused by exogenous application of ATP or noradrenaline was unaffected by the presence of sodium nitroprusside. L-NAME, L-arginine and SNAP did not affect EJP amplitude or baseline membrane potential.

It is concluded that sodium nitroprusside enhances electrically evoked contractions of the guinea-pig vas deferens by reducing the threshold voltage for action potential firing in smooth-muscle cells.

Nitric oxide synthase-immunoreactive nerve fibres are known to be present in the vas deferens of the rat (Ceccatelli et al 1994; Burnett et al 1995; Ventura & Burnstock 1996). These nerves have also been shown to be histochemically stained with nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase (Burnett et al 1995; Ventura & Burnstock 1996). NADPH-diaphorase staining of the nerves innervating the guinea-pig vas deferens has also been observed (Song et al 1994). The function of these nerves in the contractility of the vas deferens is not clear. In the rat, it has been suggested that the vas deferens does not contain nitric oxide (NO)-releasing nerves (Mitchell et al 1991). Others have discovered evidence for the participation of NO in excitatory neurotransmission in the rat vas deferens by showing that the nitric

oxide synthase inhibitor N^ω-nitro-L-arginine methyl ester (L-NAME) attenuates contractions produced by electrical-field stimulation (Vladimirova et al 1994). Similar actions of other nitric oxide synthase inhibitors such as 7-nitroindazole have been reported to be unrelated to nitrergic mechanisms in the rat vas deferens (Allawi et al 1994). The nitric oxide donor sodium nitroprusside has also been reported to have no effect on the contractions produced by exogenous application of noradrenaline or phenylephrine (Kreye et al 1975; Diamond & Janis 1978) despite the observation that it increases cyclic GMP levels in this tissue (Schultz et al 1977; Diamond & Janis 1978). Sodium nitroprusside has been reported to be both inhibitory (Schultz et al 1977) and without effect (Diamond & Janis 1978) on KCl-induced contractions of the rat vas deferens.

Less work has been conducted on the vas deferens of the guinea-pig, but NO has been shown to

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inhibit the faster-twitch response to electrical stimulation of the nerve while enhancing the secondary and slower tonic responses of long trains of stimulation (Cederqvist & Gustafsson 1994; Cederqvist et al 1994). These effects were shown to be independent of neuronal noradrenaline release. Sodium nitroprusside has also been reported to cause spontaneous contractions and enhance contractions produced by exogenous application of noradrenaline and carbachol (Sunano 1983). This excitatory action was determined to be a result of the depolarizing action of sodium nitroprusside.

The experiments using the sucrose-gap method described in this paper were performed to investigate the electrical and mechanical actions of sodium nitroprusside, and other drugs which affect nitrergic mechanisms, on the electrical and mechanical properties of the vas deferens of the guinea-pig.

Materials and Methods

Drugs
Adenosine 5'-triphosphate (ATP, disodium salt; Sigma), L-arginine (Sigma), N^{ω}-nitro-L-arginine methyl ester (L-NAME; Sigma) and sodium nitroprusside (Sigma) were all dissolved and diluted to the required concentration in Krebs solution. (−)-Arterenol bitartrate (noradrenaline; Sigma) and S-nitroso-N-acetyl-DL-penicillamine (SNAP; Alexis) were dissolved in 0·1 mM ascorbic acid and methanol, respectively, before dilution to the required concentration in Krebs solution.

Preparation of vasa deferentia
Male Dunkin-Hartley guinea-pigs (400–500 g) were killed by cervical dislocation. The abdomen was cut open and the two vasa deferentia were removed. A 1–2 cm segment from the prostatic half of the vas deferens was cut and cleared of associated blood vessels and connective tissue. The segments of the vas deferens were then cut longitudinally into two strips, yielding four preparations from each animal. Silk ligatures were tied to each end of the muscle strips for handling and the preparations were left to equilibrate in Krebs solution for 2 h before being mounted in the sucrose-gap apparatus for the recording of contractile force and electrical potential as described previously (Hoyle 1987).

In brief, a strip of smooth muscle was threaded through two rubber membranes 3 mm apart that separate a 250-μL chamber perfused with modified Krebs Bülbring solution of composition (mM): NaCl 133, KCl 4·7, NaHCO$_3$ 16·3, NaH$_2$PO$_4$ 1·4, CaCl$_2$ 2·5, MgSO$_4$ 0·6 and glucose 7·7 from a

300-μL chamber containing KCl (0·16 M). Between the membranes the tissue was bathed with sucrose (0·1 gmL^{-1}), flowing at 1 mL min^{-1}. The Krebs solution was warmed to 37°C before it entered the chamber; it was drained off by suction from the top of the meniscus. Two recording electrodes were placed in the Krebs and KCl chambers. A pair of stimulating electrodes lay horizontally in the Krebs chamber, above and below the preparation. The end of the preparation lying in the Krebs chamber was attached to an isometric force-transducer (Gould metrigram).

Tissues were stimulated electrically with trains of 10 s (60 s apart, pulse-width 0·04–0·1 ms, 100 V, 1–5 Hz). The frequency of stimulation was adjusted for each preparation so that a small contractile response could be observed. When the electrical and mechanical responses had stabilized, the superfusing Krebs solution was changed to one containing the test drug or drugs. When the responses in the presence of the test drugs had stabilized, the solution was changed back to normal Krebs solution.

Results

Responses to field stimulation
The stimulation parameters used in these experiments (10s trains of pulses every 60 s, 0·04–0·1 ms, 1–5 Hz, 100 V) elicited frequency-dependent excitatory electrical and mechanical responses (Figure 1). Electrical responses consisted of an excitatory junction potential (EJP) which accom-
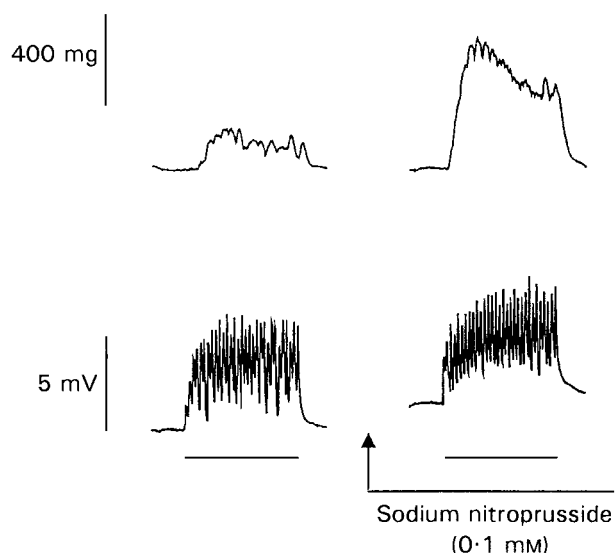

Figure 1. Typical sucrose-gap recordings of guinea-pig isolated vas deferens showing the effects of sodium nitroprusside (0·1 mM) on the mechanical (upper trace) and electrical (lower trace) responses to trains of electrical field stimulation (3Hz, 0·07 ms, 100 V, for 10 s as indicated by bars).

Table 1. Effects of sodium nitroprusside, N^ω-nitro-L-arginine methyl ester, L-arginine and S-nitroso-N-acetyl-DL-penicillamine on mean contractile force (tension) and mean fully-facilitated excitatory junction potential amplitude developed by isolated preparations of the guinea-pig vas deferens in response to trains of electric-field stimulation (1–5 Hz, 0.04–0.1 ms, 100 V, 10 s), or to application of ATP (1 mM) or noradrenaline (10 μM).

Agent	Tension (g)				Excitatory junction potentials (mV)			
	Control	Plus agent	n	P	Control	Plus agent	n	P
Electrical-field stimulation								
Sodium nitroprusside (0.1 mM)	0.19 ± 0.04	1.06 ± 0.34	8	< 0.05	3.1 ± 0.4	4.1 ± 0.9	10	NS
N^ω-nitro-L-arginine methyl ester (0.1 mM)	0.13 ± 0.05	0.16 ± 0.08	6	NS	2.3 ± 0.6	2.3 ± 0.6	6	NS
Sodium nitroprusside plus N^ω-nitro-L-arginine methyl ester	0.15 ± 0.04	0.86 ± 0.25	5	< 0.05	2.1 ± 0.4	2.4 ± 0.4	7	NS
L-Arginine (1.0 mM)	0.07 ± 0.03	0.07 ± 0.03	5	NS	3.1 ± 1.0	2.8 ± 1.0	5	NS
S-nitroso-N-acetyl-DL-penicillamine (0.1 mM)	0.20 ± 0.03	0.20 ± 0.06	5	NS	3.2 ± 0.7	3.2 ± 0.6	5	NS
Adenosine triphosphate (1 mM)								
Sodium nitroprusside (0.1 mM)	0.33 ± 0.13	0.59 ± 0.21	16	NS	2.3 ± 0.2	2.5 ± 0.6	13	NS
Noradrenaline (10 μM)								
Sodium nitroprusside (0.1 mM)	0.11 ± 0.03	0.35 ± 0.13	16	NS	1.7 ± 0.4	2.4 ± 0.6	11	NS

panied each pulse of stimulation; if the stimulation frequency was sufficiently high and the pulse duration sufficiently long, depolarization reached the threshold for action potential generation and a consequent mechanical contractile response. Because the sucrose-gap method provides a volume recording, the action potentials are manifest as small, fast, transient depolarizations of amplitude similar to that of facilitated EJPs upon which they were superimposed, but with a fast time-course (Figure 1).

Sodium nitroprusside (0.1 mM) significantly depolarized the smooth muscle membrane by 0.7 ± 0.3 mV ($P < 0.001$, one-tailed t-test, n = 35) but had no effect on the resting tension of the tissue. Sodium nitroprusside (0.1 mM) potentiated the amplitude of contractions evoked by electrical field stimulation ($P = 0.037$, paired t-test, 7 df; Table 1 and Figure 1) but did not affect EJP amplitude ($P = 0.40$, paired t-test, 9 df; Table 1 and Figure 1), although the number of action potentials in a given train of EJPs increased (Figure 1). L-NAME (0.1 mM) had no effect on resting tension or resting membrane potential, nor did it increase the magnitude of field-stimulation-induced EJPs ($P = 0.94$, paired t-test, 5 df; Table 1) or contractile responses ($P = 0.74$, paired t-test, 5 df; Table 1). Sodium nitroprusside (0.1 mM) still enhanced contractions to field-stimulation when administered in conjunction with L-NAME (0.1 mM) ($P = 0.049$, paired t-test, 4 df; Table 1), and the smooth muscle membrane was still significantly depolarized by 0.4 ± 0.2 mV ($P = 0.050$, paired t-test, 6 df). EJP amplitude was again unaffected by sodium nitro-

prusside (0.1 mM) in the presence of L-NAME (0.1 mM) ($P = 0.14$, paired t-test, 6 df; Table 1).

The NO precursor L-arginine (1 mM) and the NO donor SNAP (0.1 mM) had no effect on field-stimulation-induced contractions ($P \geq 0.89$, paired t-tests, 4 df; Table 1) nor did they affect EJP amplitude ($P \geq 0.88$, paired t-tests, 4 df; Table 1).

Responses to ATP and noradrenaline

Both ATP (1 mM) and noradrenaline (10 μM) elicited contractions in the guinea-pig vas deferens preparations. These were associated with tonic depolarizations with action potentials superimposed. Contractions to ATP (1 mM) were not affected by sodium nitroprusside (0.1 mM) ($P = 0.30$, paired t-test, 15 df; Table 1), neither were contractions induced by noradrenaline (10 μM) ($P = 0.13$, paired t-test, 6 df; Table 1). The depolarizations induced by ATP (1 mM) and noradrenaline (10 μM) were similarly unaffected by sodium nitroprusside (0.1 mM) (ATP: $P = 0.73$, 12 df; noradrenaline: $P = 0.37$, 10 df; paired t-tests; Table 1).

Discussion

The experiments described in this paper show that sodium nitroprusside enhances contractions of the guinea-pig vas deferens induced by electrical field stimulation. The fact that sodium nitroprusside did not consistently enhance contractions induced by ATP and noradrenaline suggests that the mechanism of this enhancement might be related to transmitter release rather than a direct potentiating

action on the smooth muscle. This is in agreement with previous reports that sodium nitroprusside has no effect on responses of the rat vas deferens to noradrenaline (Kreye et al 1975; Diamond & Janis 1978) but not in agreement with an earlier report of the potentiating effect of sodium nitroprusside on noradrenaline-induced contractions in the guinea-pig vas deferens (Sunano 1983). Similarly, Cederqvist & Gustafsson (1994) showed NO to have no effect on neuronal noradrenaline release in the guinea-pig vas deferens.

Although the presence of nitric oxide synthase-containing nerves in the guinea-pig vas deferens (Song et al 1994) and the observed potentiating action of sodium nitroprusside seen in our experiments seem to indicate an excitatory role for NO in this tissue, other drugs which act on nitrergic mechanisms, e.g. L-arginine and SNAP, failed to reproduce the potentiating effects of sodium nitroprusside. Similarly, the nitric oxide synthase inhibitor, L-NAME, had no antagonistic action against sodium nitroprusside and on its own did not induce effects opposite to those of sodium nitroprusside. This suggests that the L-arginine-NO pathway is not involved because L-NAME inhibits the formation of NO and L-citrulline from L-arginine during electrically stimulated neurogenic responses or endothelium-dependent responses, and the nitric oxide precursor L-arginine is best used to reverse this type of competitive inhibition by displacement of competitive antagonists, e.g. L-NAME, of the L-arginine binding site. The ferrous nitro complex, sodium nitroprusside, on the other hand, generates NO by undergoing NADPH-catalysed conversion involving membrane-bound enzymes (Feelisch 1991; Bauer et al 1995). It is, therefore, reasonable to suggest that sodium nitroprusside is generating NO via membrane-bound enzymes whereas SNAP is unable to generate NO in this preparation because its site of production of NO is more likely to be intracellular rather than extracellular, because the generation of NO from SNAP can be catalysed by membrane fractions prepared from blood vessels (Kowaluk & Fung 1990).

Our observation of the effect of sodium nitroprusside on electrical responses is also in agreement with earlier reports. Sunano (1983) showed sodium nitroprusside to have a slight depolarizing action similar to that observed in our experiments. This might account at least in part for the potentiating effect of sodium nitroprusside. The small depolarizations induced by sodium nitroprusside would bring the resting membrane potential closer to the voltage threshold at which action potential discharge was initiated. Therefore the same EJP amplitudes beginning from the raised resting membrane potential could also initiate more action potentials and at an earlier point in the train of stimulation.

The action of sodium nitroprusside in increasing the size of electrical field-stimulation-induced contractions without altering the magnitude of the associated EJPs might also indicate that sodium nitroprusside or the NO formed from it is acting by an agonistic action at voltage-dependent Ca^{2+} channels. Bay K 8644, an agonist at voltage-dependent Ca^{2+} channels, has been previously reported to have a similar potentiating action on field-stimulation-induced contractions of the guinea-pig isolated vas deferens without affecting EJP amplitude (MacKenzie et al 1988).

In conclusion, sodium nitroprusside enhanced contractile responses evoked by neuromuscular transmission but not by exogenous ATP or noradrenaline in the guinea-pig vas deferens. The lack of effect on EJP amplitude and the depolarization of the smooth muscle membrane brought about by sodium nitroprusside suggest that it is acting at a postjunctional site, possibly involving indirect stimulation of intracellular soluble guanylate cyclase.

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