

Effects of nitric oxide on electrotonically coupled neonatal rat sympathetic preganglionic neurones *in vitro* and *in vivo*.

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❶ INTRODUCTION

Sympathetic Preganglionic Neurones (SPNs) in the thoracolumbar spinal cord represent the final pathway of sympathetic output from the central nervous system to the periphery and as such constitute an important site for integration of autonomic function. The intermediolateral cell column (IML), where the majority of SPN originate, contains an abundance of NADPH-diaphorase/NOS immunoreactive cells, (Okamura, 1995), suggesting that SPN synthesise and release NO as a chemical mediator.

A population of SPN displaying spontaneous oscillations in membrane potential have previously been described by our group (Spanswick & Logan, 1990), these oscillations giving rise to rhythmic patterns of activity. It has been demonstrated that these oscillations in membrane potential are the result of electrotonic coupling between SPN (Logan *et. al.* 1996). Oscillations consist of a fast depolarising transient followed by a larger amplitude, slower hyperpolarising phase and represent a low-pass filtered action potential discharged in neighbouring neurones. A correlation between NOS expression and connexin expresssion in the mammalian CNS has been inferred suggesting a functional relationship between NO and electrotonically coupled neurones.

In view of this, we have utilised *in vitro* and *in vivo* electrophysiological recording techniques to investigate the cellular and functional role of NO in regulating SPN excitability.

❷ METHODS

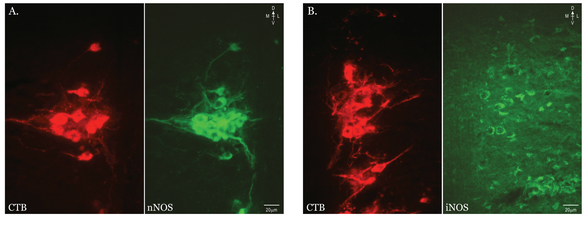
***In vitro* methods**:
Neonatal wistar-kyoto rats (6-21 days) were terminally anaesthetised with halothane (4% in O_2), cervically dislocated and decapitated and whole-cell, cell-attached and outside-out patch clamp recordings made from SPN in the intermediolateral cell column (IML) of 400 µM transverse thoracolumbar spinal cord slices as described previously (Logan *et. al.* 1996). All experiments were performed at room temperature. Junctional conductances were estimated from experimentally measured input resistances and coupling co-efficients using a model in which each neurone was represented by a single compartment joined by a resistor representing an electrical synapse (Bennett, 1996).

***In vivo* methods:**
Male adult sprague-dawley rats (300-350 g) were anaesthetised with 12% urethane. The left carotid artery, left jugular vein and trachea were cannulated for BP monitoring, intra-venous drug administration and free breathing respectively. A PE 10 tubing of about 10 cm long, filled with aCSF, was inserted into the sub-arachnoid space to the level of T9-L1 for drug application. A laparotomy was performed and the intestines reflected laterally with gauze soaked with warm saline to expose the abdominal aorta. Obstructing blood vessels were reflected to allow access to the sympathetic trunk for recording. Sympathetic trunk activity was recorded using a platinum wire electrode. Adequate depth of anaesthesia was frequently tested during the entire course of the experiment by the absence of response to firm hind paw pinch. All experiments were performed at 37 ˚C.

The following drugs were used: SNOG (S-Nitrosoglutathione), L-NMMA (NG-Monomethyl-L-arginine acetate), L-NAME (N(G)-Nitro-L-arginine methyl ester), D-NAME (N(G)-Nitro-D-arginine methyl ester), 3-Bromo-7-Nitroindazole all from Tocris Cookson; 8-Bromo-cyclic-GMP, DEA/NO (Diethylamine/nitric oxide) , L-NOARG (NG-nitro-L-arginine), D-NOARG (NG-nitro-D-arginine), 1400W (N-([3-(aminomethyl)phenyl]methyl)ethanimidamide dihydrochloride), TEA, Barium, all from Sigma Aldrich and TTX from Alomone Labs.

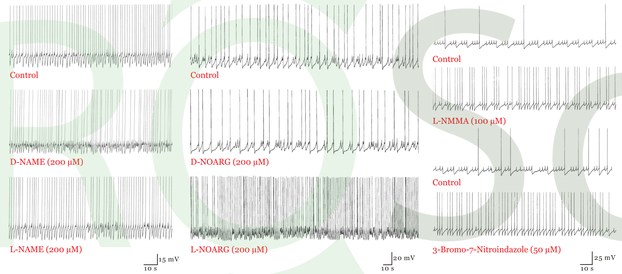
❸ SPN express NOS isoforms

A, Transverse section of thoracolumbar spinal cord showing the co-localisation of fluorescently tagged antisera directed against Cholera toxin subunit B (CTB) and nNOS.
B, Longitudinal section of thoracolumbar spinal cord showing the co-localisation, in adrenal gland innervating SPN, of fluorescently tagged antisera directed against CTB and iNOS.



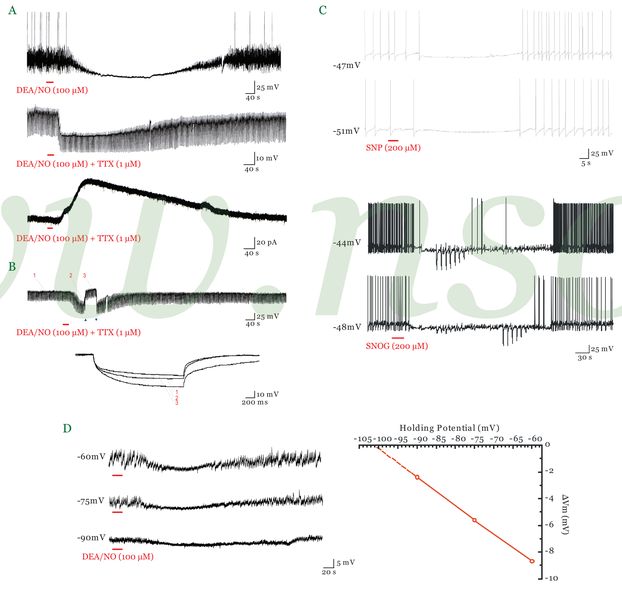
❹ NOS inhibitors increase membrane oscillations in SPN

Samples of continuous whole-cell records showing bath application of the NOS inhibitors: L-NAME (200 µM), L-NOARG (200 µM), L-NMMA (100 µM) and 3-Bromo-7-Nitroindazole (50 µM) all induced an increase in the frequency of action potential firing and membrane potential oscillations in SPN. Bath application of the inactive D-isomers of NAME and NOARG were without significat effect.



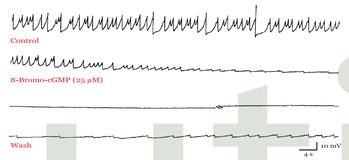
❺ NO donors supress membrane oscillations in SPN

Samples of continuous whole-cell records showing bath application of the NO donors: DEA/NO (100 µM), SNP (200 µM) and SNOG (200 µM) induced a membrane hyperpolarisation with accompanied suppression of membrane oscillations. Bath application of DEA/NO (100 µM) in the presence of TTX (1 µM), voltage clamped close to the estimated resting potential, induced an outward current. The DEA/NO induced current had a reversal potential close to -100 mV, indicative of the current being mainly carried by K+ ions.



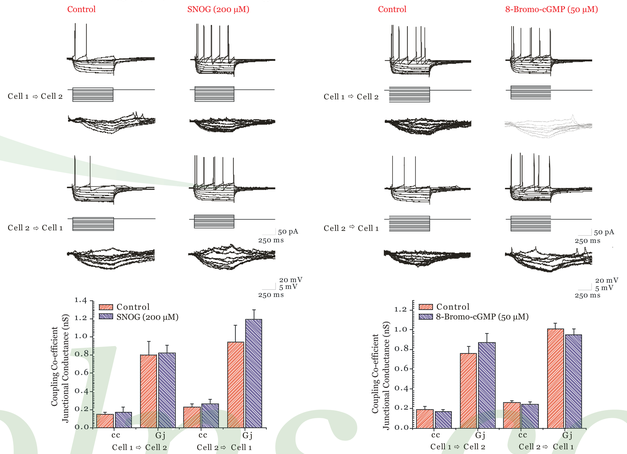
❻ cGMP analogues mimic the effects of NO donors

Application of the membrane permeable cGMP analogue, 8-Bromo-cGMP (25 µM) induced a membrane potential hyperpolarisation, accompanied by an inhibition of spontaneous membrane activity with responses being partially reversible upon wash.



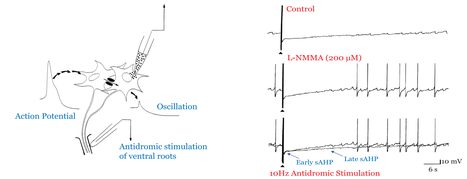
❼ NO and cGMP have little effect upon gap junctional coupling in SPN

Simultaneous recordings from a pair of electrotonically coupled SPN, demonstrating direct current transfer between cells. A series of rectangular wave current steps injected into the presynaptic cell elicited corresponding membrane potential responses in both the pre- and post-synaptic cell. Application of SNOG (200 µM) increased junctional conductance but had little effect on coupling conductance. Application of the membrane permeable cGMP analogue, 8-Bromo-cGMP (50 µM) was without significant effect.



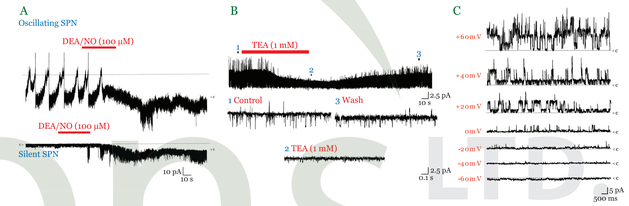
❽ Activity-dependent release of NO in SPN

Stimulation of the ventral rootlets with a bipolar stimulating electrode produced a marked after-hyperpolarisation. Following perfusion of the NOS inhibitor, L-NMMA (200 µM), the early slow afterhyperpolarisation (sAHP) decay time remained unchanged, while the late sAHP was greatly reduced. The two traces overlayed show the reduction of the late sAHP in L-NMMA.



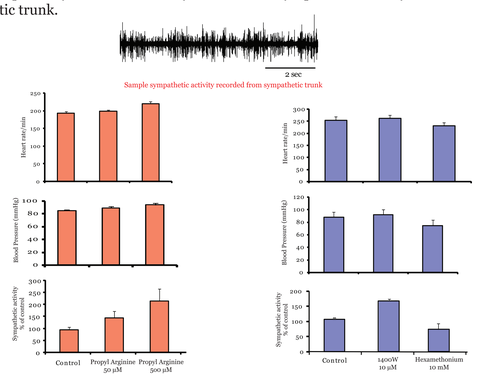
❾ NO activates ion channels

A, Cell-attached recording showing application of the NO donor, DEA/NO (100 µM) abolished membrane oscillations and activated ion channels. Channels were also activated in previously quiescent cells. B, Outside-out recording showing application of the K^+ channel blocker TEA (1 mM), blocked NO-induced channel activity. Activity returns upon wash. C, Cell-attached recording showing effects of changes in membrane potential on NO-activated channels



❿ Endogenous NO acts to inhibit sympathetic nerve discharge

Intrathecal administration of the nNOS and iNOS specific inhibitors Propyl-l-arginine and 1400w respectively both markedly increased sympathetic activity recorded from the sympathetic trunk.



⓫ CONCLUSIONS

These data suggest that NO is tonically released in a sub-population of electrotonically-coupled SPN, in an activity-dependent manner. NO, thus, acts directly to inhibit and regulate the firing frequency of SPN via an action on membrane ion channels.

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