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Complex interactions between nitric oxide and adenosine receptors in the rat isolated nodose ganglion

Andrew J. Lawrence *, Elena Krstew, Bevyn Jarrott

Department of Pharmacology, Monash University, Wellington Road, Clayton, Vic. 3168, Australia

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Abstract

The present study has employed in vitro electrophysiology, utilising the isolated rat nodose ganglion preparation, to determine whether nitric oxide (NO) and adenosine interact with each other in vagal afferent neurons. The nucleophile NO donor, diethylamine-NO, caused reproducible, concentration-related depolarisations of the isolated rat nodose ganglia. Pre-incubation of the isolated rat nodose ganglia with the adenosine A_{2A} receptor agonists CGS 21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride) and DPMA (N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine) (both 1 μM) resulted in a functional antagonism of the ability of diethylamine-NO to depolarise the preparation. A similar effect was observed with adenosine (10 μM) only in the presence of the adenosine A_1 receptor antagonist PACPX (1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine, 100 nM). Conversely, the adenosine A_1 receptor agonists ENBA (N^6-[2-endo-norbornyl]adenosine, 1 μM) and cyclohexyladenosine (100 nM) potentiated the effect of diethylamine-NO on isolated rat nodose ganglia. Inclusion of either adenosine A_3 agonists or ATP had no effect on the diethylamine-NO concentration–response curve. These data suggest an ability of NO to interact, in opposing manner, with adenosine A_{2A} and A_1 receptors in rat vagal afferent neurons. On the other hand, neither A_3 receptors nor ATP appear capable of interacting with NO.

Keywords: Nodose ganglion; Nitric oxide (NO); Adenosine; Adenosine receptor; Electrophysiology

1. Introduction

The vagus, or Xth cranial nerve, comprises largely sensory, pseudo-bipolar neurons that innervate the medulla oblongata. Ultimately included in these projections are aortic baroreceptor, cardiopulmonary and chemoreceptor afferent neurons, whose perikarya are located in the nodose ganglion and central terminals innervate the commissural and medial subregions of the nucleus tractus solitarius (Dampney, 1994; Van Giersbergen et al., 1992).

The free-radical, nitric oxide (NO) and the purine nucleoside, adenosine have both been implicated in cardiovascular control mechanisms by acting within the nucleus tractus solitarius (for a recent review see Lawrence and Jarrott, 1996). For example, microinjection of *S*-nitrosocysteine into the nucleus tractus solitarius of both anaesthetised (Lewis et al., 1991) and conscious rats (Machado and Bonagamba, 1992) results in immediate hypotension and bradycardia, mediated via the soluble guanylate cyclase effector pathway. Similarly, microinjections of adenosine into the rat nucleus tractus solitarius causes either a pressor or depressor effect, dependent upon the receptor type activated (Barraco et al., 1991). Furthermore, functional studies have demonstrated the ability of NO to depolarise rat vagal afferents (Lawrence et al., 1996) and also the presence of adenosine A_1 and A_{2A} receptors on vagal perikarya (Castillo-Meléndez et al., 1994). These observations are consistent with the presynaptic actions of NO and adenosine in the rat nucleus tractus solitarius, mediating distinct cardiovascular response patterns.

The ability of adenosine and adenosine analogues to cause relaxation of rat aortic rings involves the release of NO, and furthermore, responsiveness of aortic rings to adenosine analogues is reduced following aortic baroreceptor denervation (Fahim et al., 1994). This observation clearly links NO and adenosine with the functioning of vagal afferent neurons. Therefore, the present study has employed in vitro electrophysiology, utilising the isolated rat nodose ganglion preparation, to determine whether NO and adenosine receptors functionally interact in rat vagal afferents.

* Corresponding author. Tel.: (61-3) 9905-4855; Fax: (61-3) 9905-5851; e-mail: Andrew.Lawrence@med.monash.edu.au

2. Materials and methods

All of the experiments described here were performed in accordance with the Prevention of Cruelty to Animals Act 1986 under the guidelines of the NH&MRC Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

2.1. In vitro electrophysiology

The stable, nucleophile NO donor, diethylamine-NO (Maragos et al., 1991) was employed as the source of NO. The ability of diethylamine-NO to depolarise rat vagal afferents has been previously characterised (Lawrence et al., 1996). Electrophysiological experiments were performed as previously described (Castillo-Meléndez et al., 1994; Lawrence et al., 1995). In brief, male Sprague-Dawley rats (250–350 g) were killed by cervical dislocation and the nodose ganglia with attached vagal trunk were removed and desheathed. The tissue was then placed in a twin chambered Perspex bath with the nodose ganglion placed in one compartment of the bath and isolated from the vagal nerve trunk in an adjacent compartment by a silicone grease seal, as previously described (Widdop et al., 1990). The preparation was superfused with Krebs buffer (36°C, 2 ml/min) of the following composition (in mM): NaCl 118, $NaHCO_3$ 24.9, KH_2PO_4 1.3, KCl 4.7, $CaCl_2$ 2.6, glucose 11, $MgSO_4$ 1.2, gassed with 95% O_2/5% CO_2, pH approximately 7.4. The d.c. potential between the two compartments following drug administration to the nodose ganglion was recorded by calomel electrodes connected to the preparation through agar-KCl bridges. The potential changes were amplified and displayed on a Grass Polygraph (Model 79D). Drugs were applied non-cumulatively and remained in contact with the tissue until apparent equilibrium was reached. This was followed by a washout and recovery period (10–20 min) to allow full repolarization prior to another drug addition. When used, adenosine receptor agonists were added to the superfusate 15–20 min prior to the second concentration–response curve to diethylamine-NO application. If an adenosine receptor antagonist was employed in addition to adenosine, the antagonist was in contact with the tissue for 5 min before adenosine and remained present throughout the rest of the experimental time period. This method allowed stable responses to be measured over a 5–6 h period. The magnitude of an observed depolarization was measured from a projection of the baseline preceding the response to account for any drift in the preparation. At the beginning and end of each experiment a positive control of a previously determined maximal concentration of 5-hydroxytryptamine (5-HT, 3 μM) (Widdop et al., 1992) was applied to the nodose ganglia in order to check the viability of the preparations.

2.2. Statistical analysis

Concentration–response curves to diethylamine-NO were analysed by paired *t*-tests comparing the effect of diethylamine-NO in the absence and presence of adenosine receptor agonists. In all cases, $P < 0.05$ was considered significant.

2.3. Materials

5-Hydroxytryptamine creatinine sulphate, adenosine, and adenosine triphosphate sodium salt (ATP) were all obtained from Sigma (St. Louis, MO, USA). Cyclohexyladenosine (CHA), PD-125944 (DPMA; N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine), CGS 21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine hydrochloride), ENBA (N^6-

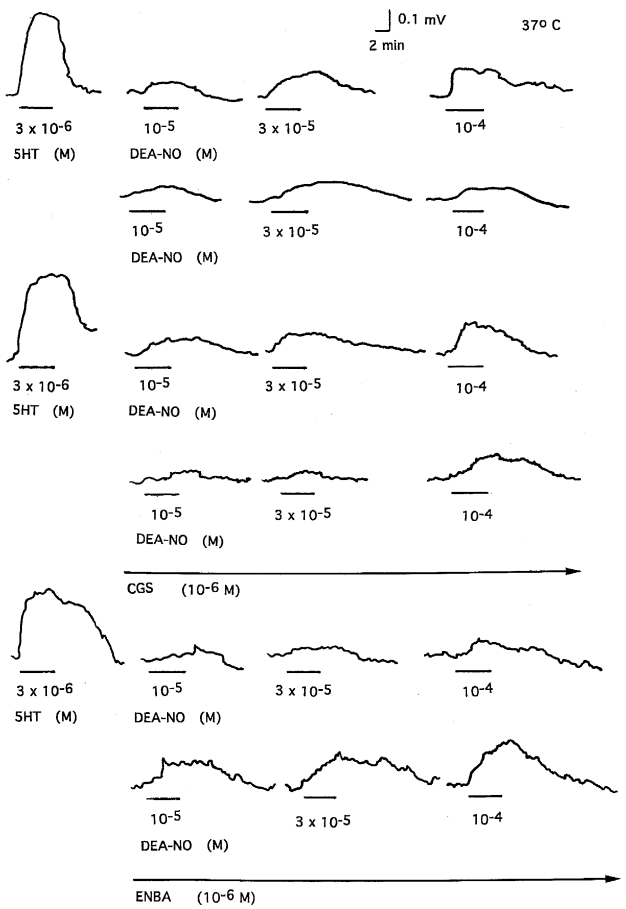

Fig. 1. Polygraph traces showing representative series of responses to different concentrations of diethylamine-NO applied to the rat isolated nodose ganglion preparation. The first two rows show control concentration-response curves to diethylamine-NO over the range shown. The middle two rows show a concentration-response to diethylamine-NO before and after a 20 min incubation of the tissue with CGS 21680 (1 μM) which was then present throughout the rest of the experiment. The bottom two rows show a concentration-response to diethylamine-NO before and after a 20 min incubation of the tissue with ENBA (1 μM) which was then present throughout the rest of the experiment. Each pair of traces is preceded by a positive control of 5-HT (3 μM). Scales as shown. Drug superfusion periods are represented by the solid horizontal bars.

[2-endo-norbornyl]adenosine), benzyl-NECA (N^6-benzyl-5′-*N*-ethylcarboxamidoadenosine) and PACPX (1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine) were all obtained from Research Biochemicals International (Natick, MA, USA). APNEA (N^6-2-(4-aminophenyl)ethyladenosine) was a gift from Dr. Steve Alexander (Nottingham University, Nottingham, UK). All other reagents were either analytical or laboratory grade from various suppliers.

3. Results

As previously demonstrated (Lawrence et al., 1996), addition of diethylamine-NO to the superfusate bathing the isolated rat nodose ganglia resulted in concentration-dependent depolarisations (Fig. 1). Furthermore, the response to diethylamine-NO has been shown to be mediated via activation of the guanylate cyclase effector pathway, and not from decomposition products (Lawrence et al., 1996). The ability of diethylamine-NO to depolarise the isolated rat nodose ganglion was reproducible; however, the response of the highest concentration of diethylamine-NO (100 μM) was diminished in the second concentration–response curve of a preparation compared to the first curve (Fig. 2A). When the preparation was exposed to the selective adenosine A_{2A} receptor agonist, CGS 21680 (1 μM), the ability of diethylamine-NO to depolarise the rat nodose ganglion was markedly reduced (Figs. 1 and 2). Analysis of concentration–response curves for diethylamine-NO in the absence and presence of adenosine A_2 agonists was made by paired *t*-tests of the diethylamine-NO response before and after adenosine agonists. Such a strategy indicated that CGS 21680 (1 μM) significantly reduced the ability of diethylamine-NO to depolarise the isolated rat nodose ganglia at concentrations of 30 and 100 μM ($P < 0.05$, $n = 4$, Fig. 2B). In a similar manner, another adenosine A_{2A} receptor agonist, DPMA (1 μM) also functionally antagonised the response of diethylamine-NO, again reducing the efficacy of diethylamine-NO at concentrations of 30 and 100 μM ($P < 0.05$, $n = 3$, Fig. 2C). In addition, a similar effect was observed when adenosine (10 μM), following blockade of adenosine A_1 receptors by PACPX (100 nM), was incubated prior to diethylamine-NO (Fig. 2D). Under these conditions, the functional antagonism of diethylamine-NO by adenosine was evident with a rightward shift in the concentration–response curve, reaching significance at 10 μM diethylamine-NO ($n = 5$). None of the adenosine receptor agonists or antagonists evoked a depolarisation alone, consistent with our previous findings that at 36°C, adenosine A_{2A} receptor agonists will only elicit a depolarisation in the presence of an adenosine A_1 receptor antagonist (Castillo-Meléndez et al., 1994).

In contrast to the functional antagonism of diethylamine-NO by adenosine A_{2A} receptor activation, inclusion of the adenosine A_1 receptor agonist, ENBA (1 μM)

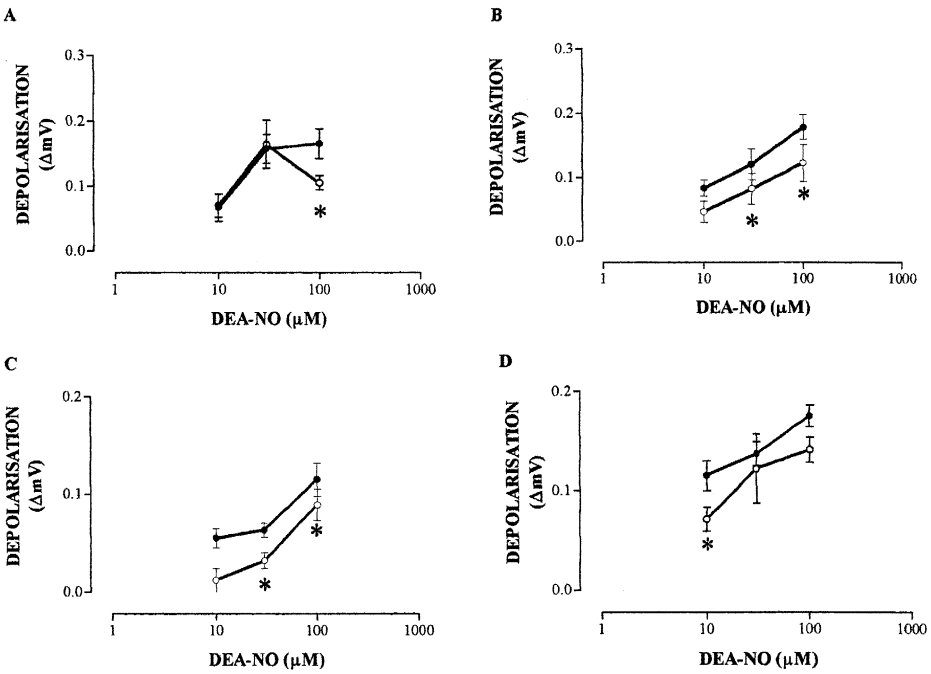

Fig. 2. Concentration–response curves of diethylamine-NO on the rat isolated nodose ganglion preparation, in the absence (closed symbols) or presence (open symbols) of: (A) nothing, control ($n = 4$), (B) CGS 21680 (1 μM, $n = 4$); (C) DPMA (1 μM, $n = 3$) and (D) adenosine (10 μM) in the presence of PACPX (100 nM, $n = 5$). Data are the mean ± S.E.M. * $P < 0.05$, paired *t*-test comparing respective point in control curve to second test curve.

in the superfusate resulted in a potentiation of the electrophysiological responses to diethylamine-NO (Fig. 3A). Thus, the efficacy of diethylamine-NO was clearly increased at concentrations of 10 and 100 μM ($n = 6$). A similar response was also observed with another adenosine A_1 receptor agonist, cyclohexyladenosine (100 nM, $n = 5$), although in this instance statistical significance was not achieved (Fig. 3B). When adenosine (10 μM, $n = 5$) was incubated with the isolated rat nodose ganglia in the absence of any selective antagonists, no significant effect was observed on the diethylamine-NO concentration–response curve (Fig. 3C). In addition, inclusion of either the adenosine A_3 agonists APNEA (1 μM, $n = 6$) or benzyl-NECA (1 μM, $n = 5$), or ATP (10 μM, $n = 4$) had no effect on the ability of diethylamine-NO to depolarise the

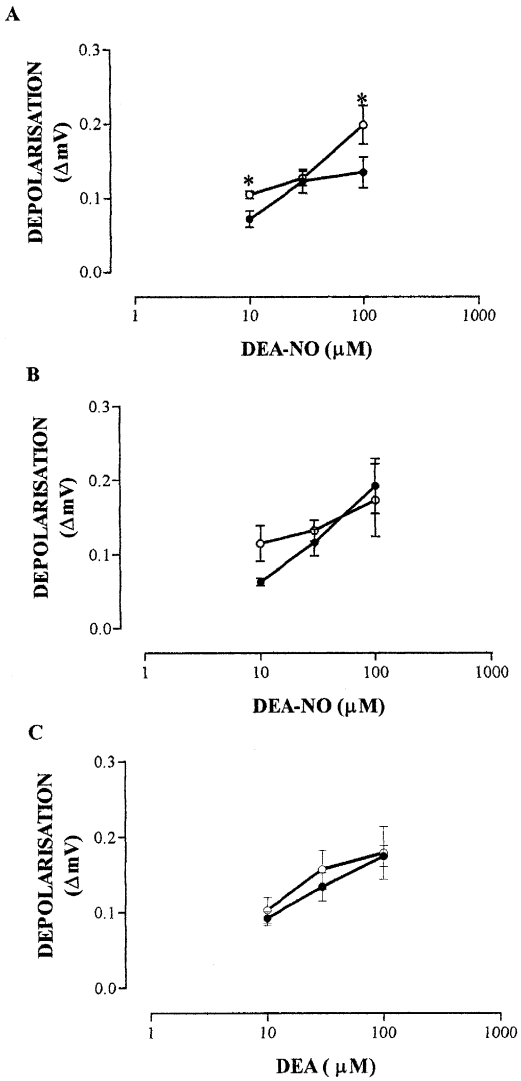

Fig. 3. Concentration–response curves of diethylamine-NO on the rat isolated nodose ganglion preparation, in the absence (closed symbols) or presence (open symbols) of: (A) ENBA (1 μM, $n = 6$), (B) CHA (100 nM, $n = 5$) and (C) adenosine (10 μM, $n = 5$). Data are the mean ± S.E.M. * $P < 0.05$, paired t-test comparing respective point in control curve to second test curve.

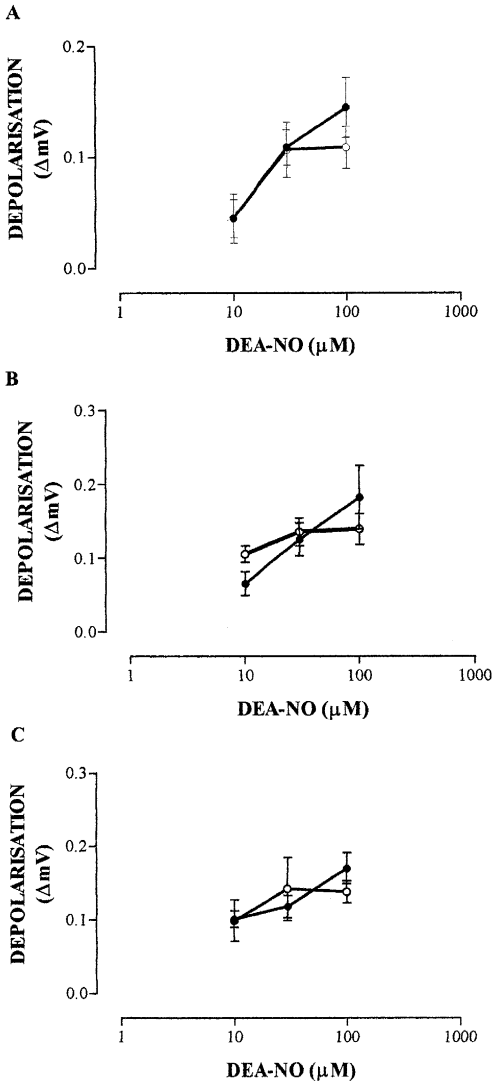

Fig. 4. Concentration–response curves of diethylamine-NO on the rat isolated nodose ganglion preparation, in the absence (closed symbols) or presence (open symbols) of: (A) APNEA (1 μM, $n = 6$), (B) benzyl-NECA (1 μM, $n = 5$) and (C) ATP (10 μM, $n = 4$). Data are the mean ± S.E.M.

preparations (Fig. 4). None of the adenosine receptor agonists or antagonists evoked a depolarisation alone.

4. Discussion

The present data provide clear evidence for both inhibitory and facilitatory interactions between adenosine and NO in rat vagal afferent neurons, dependent upon the subtype of adenosine receptor activated. Furthermore, the isolated rat nodose ganglion-vagus preparation has been developed as a method to indicate the likely presence and properties of receptors on inaccessible central vagal terminals (Round and Wallis, 1986) and therefore the data presented herein may, by analogy, represent evidence for a physiologically relevant interaction between adenosine and

NO at central vagal terminals within the nucleus tractus solitarius.

While the isolated rat nodose ganglion preparation provides simply a population response, with no indication of neuron-type, there are a multiplicity of reasons to suggest that the present observations may relate to cardiorespiratory neurons. Thus, the mRNA encoding both NO synthase (Lawrence et al., 1996) and the adenosine A_{2A} receptor (Weaver, 1993) are expressed in a restricted population of vagal perikarya, and both NO and adenosine A_{2A} receptor agonists are capable of acting presynaptically within the rat nucleus tractus solitarius to modulate the release of glutamate (Castillo-Meléndez et al., 1994; Lawrence and Jarrott, 1993), the apparent primary transmitter of baroreceptor afferents (Lawrence and Jarrott, 1994; Ohta et al., 1996; Talman et al., 1980), and thereby mediate a depressor response (Barraco et al., 1991; Lewis et al., 1991). Therefore, the functional interactions following either adenosine A_{2A} or A_1 receptor activation on the effect of diethylamine-NO may represent a regulatory process in vagal afferent neurotransmission. Whether such interactions are tonically active, or become significant during pathophysiological states, such as hypertension, remains to be determined. Interestingly, the haemodynamic responses to microinjection of adenosine into the nucleus tractus solitarii are attenuated in spontaneously hypertensive rats (Abdel-Rahman and Tao, 1996; Tseng et al., 1995). On the other hand, due to the ability of NO to diffuse trans-synaptically (Garthwaite et al., 1988), the present observations may simply represent mechanisms of inter- and intra-neuronal processing within the rat nodose ganglion.

Interactions between NO and adenosine, whereby NO donors cause release of adenosine, have been previously demonstrated both in vitro (Fallahi et al., 1996) and in vivo (Fischer et al., 1995). Furthermore, NO has been implicated to be involved in adenosine A_{2A} receptor mediated vasodilatation (Abebe et al., 1995) and hypotension (Stella et al., 1995), and also to modulate adenosine A_1 receptor mediated renal vasoconstriction (Barrett and Droppleman, 1993). The present findings therefore add to this literature, and indicate a further site of NO-adenosine interactions; however, the lack of interaction between diethylamine-NO and adenosine in the absence of an adenosine A_1 receptor antagonist may question the physiological significance of the present observations.

Recently, functional interactions between adenosine and dopamine have been characterised in rat vagal afferent neurons, whereby adenosine A_{2A} receptor activation reduced the efficacy of dopamine as a depolarising agent (Lawrence et al., 1997). One may therefore speculate that NO, adenosine and dopamine may all functionally interact to modulate neurotransmission of rat vagal afferent neurons. Given that the vagus nerve is comprised predominantly of sensory neurons, interactions between NO and adenosine (and possibly dopamine) may relate to the 'fine-tuning' of sensory inputs to the medulla oblongata, and as such provide a novel target for modulation of homeostatic processes. For example, disease states may result from either an excessive or diminished interaction, resulting in over or under stimulation of specific neuronal pathways. Whether such a hypothesis is correct clearly requires further experimentation.

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References

Abdel-Rahman, A.A., Tao, S.Y., 1996. Differential alteration of neuronal and cardiovascular responses to adenosine microinjected into the nucleus tractus solitarius of spontaneously hypertensive rats. Hypertension 27, 939–948.

Abebe, W., Hussain, T., Olanrewaju, H., Mustafa, S.J., 1995. Role of nitric oxide in adenosine receptor-mediated relaxation of porcine coronary artery. Am. J. Physiol. 269, H1672–H1678.

Barraco, R.A., El-Ridi, M.R., Ergene, E., Phillis, J.W., 1991. Adenosine receptor subtypes in the brainstem mediate distinct cardiovascular response patterns. Brain Res. Bull. 26, 59–84.

Barrett, R.J., Droppleman, D.A., 1993. Interactions of adenosine A_1 receptor-mediated renal vasoconstriction with endogenous nitric oxide and ANG II. Am. J. Physiol. 265, F651–F659.

Castillo-Meléndez, M., Krstew, E., Lawrence, A.J., Jarrott, B., 1994. Presynaptic adenosine A_{2a} receptors on soma and central terminals of rat vagal afferent neurons. Brain Res. 652, 137–144.

Dampney, R.A.L., 1994. Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 74, 323–364.

Fahim, M., El-Mas, M.M., Abdel-Rahman, A.A., Mustafa, S.J., 1994. Influence of aortic baroreceptor denervation on adenosine receptor-mediated relaxation of isolated rat aorta. Eur. J. Pharmacol. 254, 183–191.

Fallahi, N., Broad, R.M., Jin, S., Fredholm, B.B., 1996. Release of adenosine from rat hippocampal slices by nitric oxide donors. J. Neurochem. 67, 186–193.

Fischer, H., Prast, H., Philippu, A., 1995. Adenosine release in the ventral striatum of the rat is modulated by endogenous nitric oxide. Eur. J. Pharmacol. 275, R5–R6.

Garthwaite, J., Charles, S.L., Chess-Williams, R., 1988. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336, 385–388.

Lawrence, A.J., Jarrott, B., 1993. Nitric oxide increases interstitial excitatory amino acid release in the rat dorsomedial medulla oblongata. Neurosci. Lett. 151, 126–129.

Lawrence, A.J., Jarrott, B., 1994. L-Glutamate as a neurotransmitter at baroreceptor afferents: evidence from in vivo microdialysis. Neuroscience 58, 585–591.

Lawrence, A.J., Jarrott, B., 1996. Neurochemical modulation of cardiovascular control in the nucleus tractus solitarius. Prog. Neurobiol. 48, 21–53.

Lawrence, A.J., Krstew, E., Jarrott, B., 1995. Functional dopamine D_2 receptors on rat vagal afferent neurons. Br. J. Pharmacol. 114, 1329–1334.

Lawrence, A.J., Krstew, E., Jarrott, B., 1996. Actions of nitric oxide and expression of the mRNA encoding nitric oxide synthase in rat vagal afferent neurons. Eur. J. Pharmacol. 315, 127–133.

Lawrence, A.J., Krstew, E., Jarrott, B., 1997. Adenosine-dopamine receptor interactions in the isolated rat nodose ganglion but not in membranes of dorsal vagal complex. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 303–308.

Lewis, S.J., Ohta, H., Machado, B.H., Bates, J.N., Talman, W.T., 1991. Microinjection of *S*-nitrosocysteine into the nucleus tractus solitarii decreases arterial pressure and heart rate via activation of soluble guanylate cyclase. Eur. J. Pharmacol. 202, 135–136.

Machado, B.H., Bonagamba, L.G.H., 1992. Microinjection of *S*-nitrosocysteine into the nucleus tractus solitarii of conscious rats decreases arterial pressure but L-glutamate does not. Eur. J. Pharmacol. 221, 179–182.

Maragos, C.M., Morley, D., Wink, D.A., Dunams, T.M., Saavedra, J.E., Hoffman, A., Bove, A.A., Isaac, L., Hrabie, J.A., Keefer, L.K., 1991. Complexes of NO with nucleophiles as agents for the controlled release of nitric oxide. Vasorelaxant effects. J. Med. Chem. 34, 3242–3246.

Ohta, H., Li, X., Talman, W.T., 1996. Release of glutamate in the nucleus tractus solitarii in response to baroreflex activation in rats. Neuroscience 74, 29–37.

Round, A., Wallis, D.I., 1986. The depolarizing action of 5-hydroxytryptamine on rabbit vagal afferent and sympathetic neurones in vitro and its selective blockade by ICS 205-930. Br. J. Pharmacol. 88, 485–494.

Stella, L., Berrino, L., Filipelli, A., De Novellis, V., Rossi, F., 1995. Nitric oxide participates in the hypotensive effect induced by adenosine A_2 subtype receptor stimulation. J. Cardiovasc. Pharmacol. 25, 1001–1005.

Talman, W.T., Perrone, M.H., Reis, D.J., 1980. Evidence for L-glutamate as the neurotransmitter of baroreceptor afferent nerve fibers. Science 209, 813–815.

Tseng, C.J., Ger, L.P., Lin, H.C., Tung, C.S., 1995. Attenuated cardiovascular response to adenosine in the brain stem nuclei of spontaneously hypertensive rats. Hypertension 25, 278–282.

Van Giersbergen, P.L., Palkovits, M., De Jong, W., 1992. Involvement of neurotransmitters in the nucleus tractus solitarii in cardiovascular regulation. Physiol. Rev. 72, 789–823.

Weaver, D.R., 1993. A_{2A} adenosine receptor gene expression in developing rat brain. Mol. Brain Res. 20, 313–327.

Widdop, R.E., Krstew, E., Jarrott, B., 1990. Temperature dependence of angiotensin II-mediated depolarisation of the rat isolated nodose ganglion. Eur. J. Pharmacol. 185, 107–111.

Widdop, R.E., Krstew, E., Jarrott, B., 1992. Electrophysiological responses of angiotensin peptides on the rat isolated nodose ganglion. Clin. Exp. Hypertens. 14, 597–613.