Introduction

Successful communication between the two limbs of the autonomic nervous system has been inextricably linked to our evolution and survival during physiological stress (e.g. exercise, fight-and-flight response). This communication is particularly important in the heart. Abnormalities in sympathetic and parasympathetic signalling have been implicated in the aetiology and progression of cardiac dysfunction. Measurement of autonomic activity is a good independent indicator of clinical outcome (La Rovere & Schwartz, 1997), and a slowed heart rate recovery immediately after exercise is a powerful predictor of mortality (Cole et al. 1999). Impaired regulation of cardiac autonomic activity may therefore not only mirror cardiac dysfunction but actively contribute to the aetiology of the dysfunction. This lecture will touch on some of the factors that may be important in peripheral cardiac autonomic regulation during physiological and pathophysiological stress.

High intensity exercise presents one of the major chemical challenges to the myocardium (arterial plasma potassium, 8 mM, arterial pH 6.9, 15-fold increase in circulating catecholamines with local cardiac release of noradrenaline estimated at 0.5–1 µM; for review see Paterson, 1996). Protection of the heart from the chemical consequences of strenuous exercise or the mechanism by which they might trigger exercise-induced arrhythmia (Tofler et al. 1990; Mittleman et al. 1993) is not firmly established, although there are clearly critical interactions among cardiac autonomic transmitters and metabolites/ions released from contracting muscle (Paterson, 1996). Maintaining cardiac performance during exercise appears to be dependent on the cardiac autonomic nervous system and the by-products of muscle contraction interacting in such a way that each offsets the other’s potentially harmful action on the ventricle. Activation of the sympathetic nervous system, and non-adrenergic hormones (e.g. angiotensin II; Ryan & Paterson, 1996), can minimise the harmful effects of simulated exercise-induced hyperkalaemia and acidosis at the level of the single ventricular myocyte (Paterson et al. 1993), isolated perfused heart (Paterson et al. 1992; Leitch & Paterson, 1994) and whole animal (Paterson et al. 1992; O’Neill et al. 1993; O’Neill & Paterson, 1995). Stimulation of the inward calcium current in ventricular cells is essential for propagation of the action potential in raised extracellular potassium Tyrode solution (Paterson et al. 1993), and this probably accounts for the protective effect of sympathetic activation. However, ‘exercise-induced’ hyperkalaemia may also be beneficial in preventing the potential pro-arrhythmogenic action of high sympathetic activity on ventricular cells by activating the inwardly rectifying potassium current $I_{K1}$, thereby minimising a prolonged action potential duration and the

**Cardiac sympathetic imbalance and arrhythmia**

Although high adrenergic tone and hyperkalaemia are generally well tolerated by the heart during exercise (Paterson, 1996), regional cardiac ischaemia and focal sympathetic imbalance breaks down this tolerance during simulated ‘chemical exercise’ (O’Neill et al. 1995, 1997). Cardiac sympathetic imbalance can cause triggered automaticity that results in hypotension (Podzuweit et al. 1989; Nash et al. 1998a, 1999, 2001; see Fig. 1). Processes underlying this arrhythmia can be replicated using a 2-dimensional sheet of 256 by 256 resistively coupled ventricular cells, where calcium handling is abnormally high in the central region of the network (Fig. 2). Analysis of the ionic behaviour from a single cell model with upregulated Ca²⁺ conductance reveals that abnormal activation is a consequence of enhanced Na⁺–Ca²⁺ exchange which leads to high intracellular sodium initiating spontaneous depolarisation (Fig. 3). This probably resulted in a new pacemaker developing at the site of high adrenergic tone since the earliest epicardial activation always occurred at the site of the infusion (see Fig. 1; Nash et al. 2001). This relatively simple mathematical simulation illustrates the potential power of an integrative experimental-computational approach to elucidate cellular mechanisms underlying arrhythmogenesis.

Interventions that reduce spatial inhomogeneities of intracellular calcium in the ventricle may prevent focal adrenergic-induced arrhythmia. Experimentally, the arrhythmia in Fig. 1 is abolished by propranolol and vagal stimulation (O’Neill et al. 1995; Nash et al. 2001). It has been known for some time that cholinergic activation exerts an anti-arrhythmic effect on the ventricle, especially against a

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**Figure 1**

Epicardial activation sequences superimposed on 2-dimensional mathematical model of the ventricles during normal sinus rhythm (A), noradrenaline (NA)-induced ventricular arrhythmia (B) and recovery (C). Earliest epicardial activation (circles) shifted from the apical regions of the right ventricular (RV) free wall during control conditions to the NA infusion site (thick arrow), near the apex of the left ventricular (LV) free wall, during the arrhythmia. Latest epicardial activation (star) occurred adjacent to the posterobasal portions of the interventricular septum under control conditions, but moved to the posterobasal region of the LV free wall during the arrhythmia. At the onset of the arrhythmia, the QRS complex of the ECG inverted and activation time virtually doubled; this was immediately followed by a dramatic decrease in the arterial blood pressure (ABP). All changes were fully reversed after the infusion was stopped. Thick black lines denote the left anterior descending (LAD) and posterior descending (PDA) coronary arteries (modified from Nash et al. 1998b, 2001).
background of high sympathetic stimulation (Vanoli et al. 1991; Stramba Badiale et al. 1991; Hull et al. 1994). This is thought to occur through the indirect effect of muscarinic receptor activation decreasing L-type calcium currents ($I_{Ca,L}$) via inhibition of the adenylate cyclase-cyclic AMP system (Hartzell, 1988), and via activation of the nitric oxide-cyclic GMP pathway (Fig. 4) (Han et al. 1994, 1995, 1996, 1998). Whether the anti-arrhythmic effects of vagal activation against sympathetic-induced arrhythmias is mediated via an nitric oxide (NO)-cGMP-dependent pathway has not been established.

Endogenous NO is a fundamental intra/intercellular signalling molecule in the cardiovascular system (R. F. Furchgott, L. J. Ignarro & F. Murad; 1998 Nobel Prize in Medicine or Physiology). It is an important mediator of endothelial control of vascular tone, regulation of ATP production and cardiac contractile performance (e.g. Kelly et al. 1996; Shah & MacCarthy, 2000). Until recently little was known about its role as a signalling molecule in the autonomic control of cardiac excitability in the peripheral nervous system. However, its overall importance in this control is controversial, with opinion ranging from no effect through to mediation of response (see Balligand, 1999; Vandecasteele et al. 1999; Hare & Stamler, 1999).

**Nitric oxide–cGMP pathway and the cholinergic modulation of cardiac excitability**

In most species including man, endothelial nitric oxide synthase (eNOS) is expressed in cardiac myocytes from the atria, atrio-ventricular node and ventricle (Kojda et al. 1997), whereas neuronal NOS (nNOS) has been identified in both...
cholinergic and sympathetic nerve terminals (Klimaschewski et al. 1992; Tanaka et al. 1993). Balligand et al. (1993) first reported that the NOS inhibitor N\(^4\)-monomethyl-L-arginine (l-NMMA) and methylene blue blocked the negative chronotropic effects of cholinergic agonists in spontaneously beating rat neonatal myocytes. This idea was supported by Han et al. (1994, 1996), who showed that inhibition of NOS could prevent cholinergic inhibition of \(I_{\text{Ca,L}}\) without affecting the atrial muscarinic-activated \(K^+\) current, \(I_{\text{K,ACH}}\), in adrenergically pre-stimulated sino-atrial node cells and atrio-ventricular node cells. Moreover, this inhibition was absent in eNOS knockout mice (Han et al. 1998) which led them to speculate that NO played an obligatory role in the autonomic control of heart rate. However, others failed to confirm this result (Vandecasteele et al. 1998), and more recently a normal response in eNOS knockout mice to cholinergic and \(\beta\)-adrenergic activation of the inward calcium current has been reported (Vandecasteele et al. 1999; Belevych & Harvey, 2000). Some of the reasons for these differences have been discussed in detail elsewhere (Balligand, 1999). In addition, there could be some redundancy of NOS expression where nNOS might substitute for eNOS, as it does in blood vessels (Meng et al. 1998), and compensate for the removal of one of the constitutive NOS genes.

Functional data using nNOS inhibitors and non-isoform specific NOS inhibitors are as controversial as the cellular data. Conlon & Kidd (1999) reported that inhibition of nNOS caused a dramatic reduction in vagally mediated bradycardia in the adult ferret and guinea-pig \textit{in vivo}, whereas my group has reported no significant effect in the young guinea-pig \textit{in vivo}.

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**Figure 3**

A computational model was used to quantify the differences in ventricular transmembrane potential \((V_m)\) and ionic behaviour between the control state, for which the cell model was continuously paced at 2 Hz with a twice-threshold stimulus for 2 ms \((A)\), and under high adrenergic tone (stimulated by a 5-fold elevation of the L-type calcium channel and SR calcium-ATPase uptake conductances), whilst paced as above for 50 s, after which time the stimulus was switched off and the cell exhibited automaticity \((B)\). Uregulating these conductances caused the peak cytosolic calcium concentration \([\text{Ca}^{2+}]\)) and SR calcium release flux \((J_{\text{rel}})\) to markedly increase, compared to the control state, whilst the potassium current \((I_k = I_{\text{Kr1}} + I_{\text{Kr2}} + I_{\text{Kr}})\) remained similar. This intracellular calcium overload caused the \(\text{Na}^+-\text{Ca}^{2+}\) exchanger current \((I_{\text{Na–Ca}})\) to reverse and depolarise the cell, thus acting as an abnormal pacemaker with a spontaneous rate of approximately 2.2 Hz (modified from Nash et al. 2001).
in vitro during vagal nerve stimulation, with or without adrenergic pre-stimulation (Sears et al. 1998a). Furthermore, no changes in absolute rate are seen in rabbits treated with NOS inhibitors, apart from slowed transients during vagal stimulation in adrenergically pre-stimulated hearts, suggesting a small modulatory role for NO in the indirect cholinergic control of heart rate (HR), i.e. accentuated antagonism (Sears et al. 1998b). The absence of a significant

Figure 4
NO-cGMP pathway illustrating sites for pharmacological activation and inhibition.

Figure 5
A, representative raw data trace showing the effect of neuronal NOS inhibition (100 μM L-vinyl-N5-(1-imino-3-butenyl)-L-ornithine (L-VNIO)) and its reversal with 1 mM L-arginine on the heart rate response (beats min⁻¹) to vagal nerve stimulation (5 Hz, 10 V, 1 ms pulse width, 30 s duration) in a double atrial–right vagal nerve preparation from an adult guinea-pig. B, frequency–response graph for the decrease in heart rate (beats min⁻¹) with right vagal nerve stimulation (1, 3 and 5 Hz) in atria from adult guinea-pigs (n = 7). Neuronal NOS inhibition (100 μM L-VNIO) significantly attenuated (*P < 0.05) the negative chronotropic responses at 3 and 5 Hz and this effect was significantly reversed by 1 mM L-arginine (**P < 0.05). C, nNOS inhibition (100 μM L-VNIO) had no effect on the heart rate response (beats min⁻¹) to cumulative doses of carbamylcholine in adult guinea-pig atria (n = 5) (modified from Herring et al. 2000a).
effect in the rabbit has also been observed by others (Liu et al. 1996; Conlon & Kidd, 1999). However, the discrepancy in response amongst species may be related to the developmental state of the tissue being studied. Herring et al. (2000a) have reported that whilst nNOS and guanylyl cyclase inhibitors do not alter the HR response to vagal nerve stimulation in isolated young guinea-pig atria, these agents significantly inhibit the HR response to vagal stimulation in older animals (Figs 5 and 6) that express significantly more nNOS protein (Fig. 7). This suggests that the levels of NOS expression and NO bioavailability are directly coupled to developmental state. Conversely, by-passing endogenous NOS and amplifying NO production with NO donors or its second messenger with 8-bromo-cGMP enhances the drop in HR caused by vagal nerve stimulation in vitro and in vivo, irrespective of developmental state (Sears et al. 1999; Herring et al. 2000a).

When the stable analogue of ACh, carbamylcholine, is applied in the presence of NOS and guanylyl cyclase inhibitors (Figs 5 and 6) and NO activators, there is no effect on HR (Sears et al. 1999; Herring et al. 2000a), consistent with a presynaptic modulation of vagal neurotransmission. Indeed Neil Herring from my group (unpublished observation and Herring et al. 2000b) has recently shown that sodium nitroprusside facilitates the release of 3H-acetylcholine during field stimulation in guinea-pig atria via a presynaptic guanylyl cyclase-dependent pathway. This probably results in cGMP stimulation of phosphodiesterase 3 (PDE3) that increases cAMP–protein kinase A (PKA)-dependent phosphorylation of N-type calcium channels. Inhibition of PDE3 with milrinone, of PKA with H89, and of N-type calcium channels with ω-conotoxin all inhibit the HR response to NO donors during vagal activation (Herring et al. 2000b). When these results are taken together, they support the idea the NO–cGMP-dependent pathway can activate neuronal Ca²⁺

Figure 6

A, representative raw data trace showing the effect of guanylyl cyclase inhibition (10 µM 1H-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one (ODQ)) on the heart rate response (beats min⁻¹) to vagal nerve stimulation (5 Hz, 10 V, 1 ms pulse width, 30 s duration) in a double atrial–right vagal nerve preparation from an adult guinea-pig. B, frequency–response graph for the decrease in heart rate (beats min⁻¹) with right vagal nerve stimulation (1, 3 and 5 Hz) in atria from adult guinea-pigs (n = 7). Guanylyl cyclase inhibition (with 10 µM ODQ) significantly attenuated the negative chronotropic responses at 3 and 5 Hz (*P < 0.05). C, guanylyl cyclase inhibition (10 µM ODQ) had no effect on the heart rate response (beats min⁻¹) to cumulative doses of carbamylcholine in adult guinea-pig atria (n = 6) (modified from Herring et al. 2000a).
channels that promote calcium entry and exocytotic release of neurotransmitter. In conscious nNOS knockout mice, baseline HR is high and the HR response to atropine is blunted compared to wild-type controls (Jumrussirikul et al. 1998). Moreover, in the isolated atria–vagal preparation from the nNOS−/− mouse, the HR response to vagal nerve stimulation, but not to carbachol, is reduced compared to wild-type litter mates. This further suggests that neurally generated NO can modulate ACh release during vagal activation (Choate et al. 2000). In humans, NO donors have been shown to increase the high frequency component of heart rate variability, an index of cardiac vagal tone (Chowdhary et al. 2000), although others do not see this (Hogan et al. 1999b).

The NO–cGMP pathway is not the only modulator of transmitter release and HR during vagal activation. Inhibition of sulphonylurea-sensitive K⁺ channels (KATP channels) also facilitates the release of ACh (Fabiani & Story, 1995) and bradycardia during vagal nerve stimulation (Almond & Paterson, 2000). In mesenteric arteries NO hyperpolarises the cell membrane by opening KATP channels, whereas dilatation in coronary arterioles by NO donors or 8-bromo-cGMP is unaffected by inhibition of KATP channels (Hein & Kuo, 1999). Consistent with this latter observation, NO donors or 8-bromo-cGMP further enhance the HR response to vagal nerve stimulation in the presence of a maximal inhibiting concentration of sulphonylurea, suggesting that the two pathways can act independently (Almond & Paterson, 2000).

NO is tonically released in the heart, reaching concentrations of 1–3 µM during diastole (Pinsky et al. 1997). In addition to its presynaptic action, NO has a significant postsynaptic action via ACh–M₂ receptor coupling to eNOS (Balligand et al. 1993; Balligand, 1999). NO-sensitive neurons are also present in stellate and intrinsic cardiac ganglia. These neurons can generate NO and increase beating rate when co-cultured with cardiac myocytes (Horackova et al. 1995; Armstrong et al. 1995). NOS inhibition causes a small bradycardia (Kojda et al. 1997; Musialek et al. 1999) and eNOS knockout mice have a lower basal HR that is independent of high blood pressure (Shesely et al. 1996). This suggests that NO directly stimulates basal HR. Musialek et al. (1997, 1999) found that low doses of NO donors or 8-bromo-cGMP increases spontaneous beating in isolated guinea-pig atria and rabbit sino-atrial node cells by activation of the hyperpolarisation inward current Iₑ. Functionally, the increase in HR caused by NO donors is independent of baroreflex activation since the rate response is present in isolated working rabbit hearts (with pre-load and after-load held constant; Hogan et al. 1999a), cardiac autonomically denervated and β-blocked rabbits and pigs (Hogan et al. 1999a; Musialek et al. 2000), and in humans where blood pressure has been held constant with an infusion of phenylephrine (Hogan et al. 1999b). Presynaptically, NO may facilitate ACh release to decrease HR, but postsynaptically it may stimulate HR by activating Iₑ. This latter idea is supported by functional data that show inhibition of Iₑ with 2 mM caesium chloride causes a faster drop in the HR response to vagal nerve stimulation compared to control stimulations (Sears et al. 1998b). When all data are taken together, there may be an important interplay between Iₑ and Iₑ that results in an opposing action (Sears et al. 1998b). NO may act to inhibit Iₑ (Han et al. 1994) to slow the heart rate (Sears et al. 1996; Conlon et al. 1996; Elvan et al. 1997; Han et al. 1994), but also stimulate Iₑ (Musialek et al. 1997), thereby attenuating the rapid decrease in HR caused by cholinergic activation (Sears et al. 1998b). The interplay between Iₑ and Iₑ by the NO–cGMP pathway may depend on the amount of background adrenergic activation since inhibition of NOS has little effect on β-adrenoceptor-induced inhibition of Iₑ in pacemaker cells that have not been pre-stimulated with β-adrenoceptor agonists (Han et al. 1994).

**Nitric oxide–cGMP pathway and the sympathetic modulation of cardiac excitability**

Increases in cGMP caused by NO can also interfere with β-adrenergic signalling (Kelly et al. 1996). Depending on the species, cGMP can inhibit the action of β-receptor stimulated cAMP on pacemaking (Han et al. 1994) by catalysing the catabolism of cAMP through activation of phosphodiesterase activity or inhibiting calcium channel activity via the cGMP–PKG pathway (Han et al. 1995). In addition, NO can significantly affect cardiac sympathetic neurones. Inhibition of NO synthase with non-isomorphic and specific neuronal NOS inhibitors enhances noradrenaline release (Schwarz et al. 1995) and the HR and contractile responses to cardiac sympathetic nerve stimulation in vitro (Choate & Paterson, 1999; see Fig. 8) and in vivo (Elvan et al. 1997; Sears et al. 1998a), effects that are all reversed by excess L-arginine. Similarly, intracerebroventricular injection of a NOS inhibitor causes an increase in central sympathetic outflow (Togashi et al. 1992; Zanzinger et al. 1994, 1995) and an increase in baroreceptor-dependent renal sympathetic nerve activity (Matsumura et al. 1998). Inhibition of soluble guanylyl cyclase also enhances the HR response to peripheral sympathetic nerve stimulation, whereas NO donors and 8-bromo-cGMP depresses cardiac excitability during sympathetic nerve stimulation (Choate & Paterson, 1999; see Fig. 8). The HR response to bath-applied noradrenaline or isoprenaline is not affected by NOS inhibition, suggesting that

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**Figure 7**

Western blot showing significantly lower levels of nNOS (120 kDa) protein in right atria isolated from three young compared to three adult guinea-pigs. nNOS was found at both 120 kDa and 160 kDa in guinea-pig fore-brain. Equal amounts of protein (200 µg) were loaded into each lane. (from Herring et al. 2000a).
NO can act presynaptically to inhibit transmitter release via a cGMP-dependent pathway (Sears et al. 1998a; Choate & Paterson, 1999).

The intracellular signalling pathway underlying the presynaptic release of noradrenaline by NO has not been firmly established, although it could involve activation of cGMP-dependent phosphodiesterases that have an inhibitory effect on cAMP-dependent protein kinases to decrease calcium entry and exocytotic release of transmitter (Choate & Paterson, 1999). In addition, the NO-cGMP pathway may modulate other protein kinase-activated ion channels such as K<sub>ATP</sub> channels. Activation of these channels inhibits NA release (Oe et al. 1999) and the HR response to sympathetic nerve stimulation <i>in vitro</i> (Mohan & Paterson, 2000). The response to sulphonylureas is not mimicked by bath-applied noradrenaline, suggesting a presynaptic action. In addition,

![Figure 8](image_url)

**Figure 8**

*A.* Effect of NO on the heart rate response to sympathetic nerve stimulation (SNS; 3 Hz, 15 V, 30 s) in isolated guinea-pig atria. Note that inhibition of nNOS with 7-nitro indazole (7-NiNa, 100 µM) enhances the positive chronotropic effect of SNS whereas the nitric donor sodium nitroprusside (SNP, 100 µM) inhibits it. *B.* Effect of the nNOS inhibitor 1-2-trifluromethylphenyll imidazole (TRIM, 100 µM; n = 6) on the heart response to SNS and the addition of excess L-arginine (1 mM) on the heart rate with SNS; *P* < 0.05. *C.* Effect of SNP (100 µM) and its wash-off on the heart response to SNS; *P* < 0.05 (modified from Choate & Paterson, 1999).
modulation of the HR response to sympathetic nerve stimulation by the NO–cGMP pathway and by sulphonylurea-sensitive pathways appear to be independent of each other in the isolated guinea-pig atria (Mohan & Paterson, 2000).

**Functional significance of nitric oxide in the autonomic regulation of cardiac excitability**

Nitrovasodilators release NO and are commonly used to test the sympathetic component of the baroreflex since they are thought to have no direct action on the heart and the sympathetic nervous system. However, the appropriateness of their use has recently been questioned (Casadei & Paterson, 2000), given that NO can directly stimulate pacemaking via $I_f$ (Musialek et al. 1997), inhibit both peripheral (Schwarz et al. 1995; Choate & Paterson, 1999) and central sympathetic neurotransmission (Togashi et al. 1992; Zanzinger et al. 1994, 1995) and interfere with downstream $\beta$-adrenergic signalling (Kelly et al. 1996). Therefore any quantitative interpretation of the baroreflex using nitrovasodilators must now take into account the extravascular effects of these drugs (Casadei & Paterson, 2000).

Exercise training reduces the HR response to submaximal exercise. Recent work from our group has shown that a significant component of this response occurs via a decreased peripheral presynaptic sympathetic response. This is in part related to increased expression of nNOS in sympathetic ganglia, and presumably increased bioavailability of NO inhibiting noradrenaline release, although other factors are clearly involved (Mohan et al. 2000). In pathophysiological states (e.g. myocardial ischaemia, sepsis, heart failure) that result in high cardiac adrenergic stimulation, activation of cardiac $K_{ATP}$ channels and the NO–cGMP pathway may play an important complementary role in reducing local exocytotic release of noradrenaline to help protect the heart by reducing HR and to minimise oxygen consumption and cardiac work. Activation of these pathways in vivo could be of therapeutic value if specific pharmacology could be targeted at the heart. This is important in order to minimise the vascular action of these drugs activating sympathetic reflexes caused by the baroreflex.

**Summary**

Evidence is now emerging which indicates that the NO–cGMP pathway plays an important role in the sympatho-vagal modulation of cardiac excitability at various levels in the autonomic nervous system. Recent work shows that the NO–cGMP pathway can inhibit the HR response to cardiac sympathetic stimulation by reducing the presynaptic release of NA. Conversely, the NO–cGMP pathway facilitates the release of ACh and the HR response to vagal activation. Functionally, this response predominantly occurs via presynaptic modulation of transmitter release, but the pathway may also be coupled to muscarinic receptor activation and downstream inhibition of calcium currents in pacemaking cells. Factors that amplify NO signalling may

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**Figure 9**

Summary pathway highlighting the functional consequences of the NO pathway on the cardiac autonomic modulation of HR. Amplification of NO–cGMP pathway (+NO) attenuates the HR response to sympathetic activation whereas it facilitates vagal-induced bradycardia. Conversely, inhibition of neuronal NO synthase (–NO) facilitates the HR response to SNS and attenuates the HR response to vagal nerve stimulation. These responses are not mimicked by bath-applied transmitter, suggesting that the dominant functional role of HR control is via presynaptic modulation of transmitter release.
promote sympathetic inhibition and facilitation of cholinergic activity. The reciprocal nature of this signalling pathway in the modulation of peripheral cardiac excitability is illustrated in Fig. 9.


**BELEVIN, A. E. & HARVEY, R. D. (2000).** Mucarinic inhibitory and stimulatory regulation of the L-type Ca


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