Lidocaine relaxation in isolated rat aortic rings is enhanced by endothelial removal: possible role of \( K_v \), \( K_{ATP} \) channels and \( A_{2a} \) receptor crosstalk

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Abstract

**Background:** Lidocaine is an approved local anesthetic and Class 1B antiarrhythmic with a number of ancillary properties. Our aim was to investigate lidocaine’s vasoreactivity properties in intact versus denuded rat thoracic aortic rings, and the effect of inhibitors of nitric oxide (NO), prostenoids, voltage-dependent \( K_v \) and \( K_{ATP} \) channels, membrane \( Na^+/K^+ \) pump, and \( A_{2a} \) and \( A_{2b} \) receptors.

**Methods:** Aortic rings were harvested from adult male Sprague Dawley rats and equilibrated in an organ bath containing oxygenated, modified Krebs-Henseleit solution, pH 7.4, 37 °C. The rings were pre-contracted sub-maximally with 0.3 \( \mu \)M norepinephrine (NE), and the effect of increasing lidocaine concentrations was examined. Rings were tested for viability after each experiment with maximally dilating 100 \( \mu \)M papaverine. The drugs 4-aminopyridine (4-AP), glibenclamide, 5-hydroxydecanoate, ouabain, 8-(3-chlorostyryl) caffeine and PSB-0788 were examined.

**Results:** All drugs tested had no significant effect on basal tension. Lidocaine relaxation in intact rings was biphasic between 1 and 10 \( \mu \)M (Phase 1) and 10 and 1000 \( \mu \)M (Phase 2). Mechanical removal of the endothelium resulted in further relaxation, and at lower concentrations ring sensitivity (% relaxation per \( \mu \)M lidocaine) significantly increased 3.5 times compared to intact rings. The relaxing factor(s) responsible for enhancing ring relaxation did not appear to be NO- or prostacyclin-dependent, as L-NAME and indomethacin had little or no effect on intact ring relaxation. In denuded rings, lidocaine relaxation was completely abolished by \( K_v \) channel inhibition and significantly reduced by antagonists of the MitoK ATP channel, and to a lesser extent the SarcK ATP channel. Curiously, \( A_{2a} \) subtype receptor antagonism significantly inhibited lidocaine relaxation above 100 \( \mu \)M, but not the \( A_{2b} \) receptor.

**Conclusions:** We show that lidocaine relaxation in rat thoracic aorta was biphasic and significantly enhanced by endothelial removal, which did not appear to be NO or prostacyclin dependent. The unknown factor(s) responsible for enhanced relaxation was significantly reduced by \( K_v \) inhibition, 5-HD inhibition, and \( A_{2a} \) subtype inhibition indicating a potential role for crosstalk in lidocaine’s vasoreactivity.

**Keywords:** Rat aorta, Lidocaine, Relaxation, Vasodilation, Endothelium, Nitric oxide, Redox stress
Background

Lidocaine is a local amide-type cationic anesthetic, which acts by blocking voltage-dependent Na⁺ fast channels in excitable cells (EC50, 50–100 μM) [1]. At lower concentrations, lidocaine is an approved Class 1B antiarrhythmic [2] and exerts anti-inflammatory [3], neuroprotective [4], energy-lowering [5], anti-ischemic [6, 7], anti-oxidant [8, 9] and platelet-neutrophil interactive [10, 11] properties.

Lidocaine has also been shown to exert a number of vasomodulatory properties in isolated vessels including: 1) endothelium-independent relaxation [12, 13], and 2) vascular smooth muscle relaxation [12, 14, 15] or contraction [15–19] properties. The apparent paradoxical nature of lidocaine on vascular smooth muscle is often explained as being dose-dependent with vasoconstriction of peripheral blood vessels occurring at low concentrations of lidocaine (~5 μM) and vasodilation at higher levels (>50 μM) [14, 16, 20, 21]. In the rat carotid artery, Kinoshita further proposed that lidocaine may impair the vasodilator response via the activation of ATP-sensitive K⁺ channels which may be exacerbated by hypoxia [19]. Earlier the same group showed that in precontracted denuded rat aortic rings that acidification promoted lidocaine relaxation and alkanization led to vasoconstriction [18].

These confounding effects of lidocaine vasoreactivity appear to be linked to differential modulation of multiple channels including Na⁺ channels [2], inwardly-rectifying K⁺ channels [22], Ca²⁺ channels [13, 23] and/or K_{ATP} channels [18, 19]. Vasodilatation may involve nitric oxide (NO) [24–26], redox regulation [9] and possible convergence of a multitude of downstream cAMP and cGMP signalling cascades that lead to changes in cytosolic Ca²⁺ [27]. Hollmann and colleagues, for example, identified lidocaine and G-protein coupled receptor systems as potential intracellular signalling mechanisms, and the Gq protein subunit as a possible common target [28]. In 2003, Benkwitz et al., also showed that the Gq protein subunit was enhanced by lidocaine, and that it was potentiated adenosine A1-receptor signalling [29]. The group proposed that lidocaine was not an A1-receptor agonist but enhanced adenosine-A1 receptor signalling separate from its local anesthetic Na⁺ channel properties [29]. The aim of the present study was to investigate the nature of lidocaine relaxation in isolated rat thoracic aortic rings, and examine the effect of inhibitors of NO, prostenoids, Kᵋ channels, K_{ATP} channels, and adenosine A₂a and A₂b receptors. Adenosine A₂ receptors were chosen because they are widely known to modify vascular tone [30], and may therefore be involved in possible crosstalk in lidocaine relaxation [29].

Methods

Animals

Male Sprague Dawley rats (300–350 g, n = 72) were fed ad libitum and housed in a 12-h light/dark cycle. On the day of the experiment rats were anaesthetised with Thiopentone (100 mg/kg). Animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The James Cook University Ethics approval number for the studies was A1535. Lidocaine hydrochloride was sourced as a 2% solution (Ilum) from the local Pharmaceutical Suppliers (Lyppard, Queensland). All other chemicals, drugs and inhibitors were purchased from Sigma Aldrich (Castle Hill, NSW).

Aortic ring preparation and organ bath tension measurements

The thoracic cavity of anesthetized rats was opened and the thoracic aorta was harvested and placed in a modified ice-cold solution of Krebs Henseleit (118 mM NaCl, 4.7 mM KCl, 1.2 mM Na₂PO₄, 0.5 mM MgCl₂, 1.12 mM CaCl₂, 25 mM NaHCO₃, 0.03 mM EDTA) pH 7.4 with 11 mM glucose. The aorta was carefully dissected from surrounding fat and connective tissue and cut into short transverse segments. Intact aortic rings were isolated from each rat and used without further processing. In those studies that required removal of the endothelium, intact rings were denuded by gently rubbing the intimal surface of the vessel segment with a smooth metal probe. Successful removal of the endothelium was assessed by testing the aortic ring for a vasodilatory response to 10 μM acetylcholine (final concentration).

After preparation, intact or denuded aortic rings (3 to 4 mm long) were equilibrated in a standard 10 ml volume organ bath (Radnotti Glass, ADinstruments, NSW, AUS) containing modified Krebs Henseleit (see above) and continuously bubbled with 95% O₂ and 5% CO₂ at 37 °C for 15 min (zero tension) (Fig. 1). The rings were vertically mounted on small stainless steel triangles, stirrups and connected to an isometric force transducer (PANLAB, distributed by ADInstruments as MLT 0201/RAD, NSW, AUS) coupled to a computer based data acquisition system (PowerLab, ADInstruments) and data recording software LabChart 7 (ADInstruments Pty Ltd., Castle Hill, Australia) (Fig. 1).

The ring tension was manually adjusted to 1.5 g and equilibrated for 60 min. A tension of 1.5 g was chosen from the literature for thoracic aortic rings prepared from 300 to 400 g rats [18, 31] and preliminary studies verified this tension. During equilibration, the solution was changed in 15 min intervals. The aortic rings were then washed with freshly prepared Krebs Henseleit buffer pH 7.4 and the tension was readjusted to 1.5 g
tension. Each preparation was sub-maximally contracted using 3 μl of 0.1 mM NE (0.3 μM final concentration) [15, 32]. Those aortic rings that failed to contract were discarded. Ten microliters of 10 mM acetylcholine (10 μM final concentration) was applied to confirm the presence or absence of an intact endothelium in all preparations. Acetylcholine will induce rapid relaxation of precontracted rings if the endothelium is intact and if the endothelium is removed (or denuded) the rings will remain in contracted state [33]. Aortic rings were considered intact if the relaxation induced by 10 μM ACh was greater than 80%, and the aortic ring was assumed denuded if relaxation was less than 10%.

Rings were contracted at least two more times before each experiment until a reproducible contractile response was obtained. Ten to 15 min after this state was achieved the experiment was commenced because preliminary studies showed that the increase in tension and plateau from 0.3 μM of NE was reached at 10 min and remained at this plateau level for over 60 min, the time course of each experiment.

Lidocaine relaxation in intact and denuded rat aortic rings: a scheme of the experimental protocol

Lidocaine-HCl was added into the oxygenated organ bath containing KH solution to obtain 1, 5, 10, 50, 100, 500 and 1000 μM lidocaine concentrations. The change in tension of pre-contracted intact or denuded rings was measured. Responsiveness was defined as % relaxation per μM lidocaine. The inhibitors used in this study were incubated in organ bath 20–30 min before NE was administered followed by lidocaine incremental administration. These included 1). 100 μM Nα-nitro-L-arginine Methyl Ester (L-NAME) (nitric oxide synthetase inhibitor) and 10 μM indomethacin (cyclooxygenase or prostaglandin inhibitor e.g. prostacyclin). NO and prostacyclin are two major endothelial derived relaxation factors (EDRF), and the inhibitors were only applied in endothelium intact aortic rings. 2). 1 mM 4-aminopyridine (4-AP) (Non-selective voltage-dependent K+ -channel blocker of the Kv1 to Kv4 families rather than Kv7 channels) [34–36], 10 μM glibenclamide (Non-selective SarcKATP channel blocker) [37, 38] and 1 mM 5-hydroxydecanoate (5-HD) (Non-selective MitoKATP channel blocker) [39], and Na+/K+-ATPase inhibitor (100 μM ouabain) [40]. While 5-HD is commonly used in the literature as a specific MitoKATP channel blocker [41], Hanley and colleagues have shown that 5-HD is not a selective inhibitor of mitochondrial KATP channels but can act as a substrate for the mitochondrial outer membrane enzyme acyl-CoA synthetase in the beta-oxidation pathway [42]), and it is also capable of playing a role as an inhibitor of sarcolemmal KATP channels in the presence of ATP (which was not the case in our...
study) [43]. These inhibitors were applied to intact endothelium rings in the presence of L-NAME and indomethacin, and without the presence of L-NAME and indomethacin in denuded aortic rings, and 3) The adenosine A2a receptor inhibitor, 100 μM 8-(3-chlorostyryl) caffeine (CSC) [44–46], and the A2b receptor inhibitor, 10 μM 8-(4-(4-(4-chlorobenzyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine (PSB-0788) [47]. These high affinity antagonists have been used in rodent studies with reported K_i values of 24 nM for CSC [48] and 0.393 nM for PSB-0788 [47]. CSC has also been shown to be 520-fold selective for A2a-adenosine receptors in radioligand binding assays in the rat brain (K_i, 54 nM) with little or no effect on A1 receptors [44]. The inhibitors were applied to isolated rat aortic rings in an oxygenated medium. At the end of each experiment, the rings were tested for viability (or patency) by being maximally dilated with 100 μM papaverine, and relaxation was expressed as percentage of maximal relaxation to papaverine [40, 49].

Statistics
Values are expressed as mean ± SEM. The number of rats was selected from a priori G-power analysis to achieve a level of 1.0. Values are expressed as mean ± SEM. All data was tested for normality using Kolmogorov-Smirnov test. Relaxation responses to lidocaine were analysed for homogeneity of variances followed by two-way ANOVA coupled with the Bonferroni post-hoc test for individual data point comparisons. The alpha level of significance for all experiments was set at p < 0.05.

Results
Effect of increasing lidocaine on relaxation in intact and denuded rings

Intact rings
The gram tension produced with NE administration in endothelium intact rings was not significantly different from denuded aortic rings. Lidocaine produced a concentration-dependent, biphasic relaxation relationship in intact and denuded rat aortic rings (Fig. 2). The percentage relaxation in intact rat aortic rings was 1.3, 6.0, 8.6 and 41.7% at 1, 10, 100 and 1000 μM lidocaine respectively. The first relaxation phase was between 1 and 10 μM (Phase 1) and the second phase was from 10 to 1000 μM lidocaine (Phase 2) (log concentration scale) (Fig. 2). The percentage relaxation per μM lidocaine (ring responsiveness) was 0.47% from 1 to 10 μM, 0.029% between 10 and 100 μM and 0.037% increase per μM between 100 and 1000 μM lidocaine. The maximum relaxation from 1 to 1000 μM lidocaine in intact rings was 40.4%.

Denuded rings
Removal of the endothelium significantly increased Phase 1 relaxation responsiveness from 0.47 to 1.80% per μM lidocaine between 1 and 10 μM (Fig. 2). Interestingly, above 10 μM removing the endothelium had little or no significant effect on ring responsiveness to increasing lidocaine compared to intact rings. From 10 to 100 μM, % relaxation per μM was 0.033% and from 100 to 1000 μM was 0.028% (Fig. 2). However, despite this similar responsiveness, at each lidocaine concentration up to 100 μM, the absolute percentage relaxation was significantly higher in denuded rings than intact rings. The absolute % relaxation in denuded rings was 5.5, 14, 22, 24, 25, 36 and 50% at 1, 5, 10, 50, 100, 500 and 1000 μM lidocaine respectively (Fig. 2). Thus the effect of removing the endothelium was to significantly enhance ring sensitivity or responsiveness at lower lidocaine concentrations (1 to 10 μM) but not in the higher range (10 to 1000 μM), even though absolute relaxation values were significantly higher at each lidocaine concentration (1 to 1000 μM) in denuded versus intact rings (Fig. 2).

Effect of L-NAME and indomethacin in intact aortic rings
In intact aortic rings, pre-treatment with L-NAME and indomethacin did not significantly change lidocaine relaxation from 1 to 1000 μM, although there was a trend towards inhibition at higher concentrations (Fig. 3). Between 1 and 10 μM, the change in relaxation was 0.44% per μM, 0.002% per μM between 10 and 100 μM and 0.029% per μM from 100 to 1000 μM. At 500 μM lidocaine, the % relaxation was 17% (32% lower than intact rings) and at
1000 μM lidocaine was 32% (24% lower than intact rings), but the differences were not significant (Fig. 3).

**Effect of K$_v$, SarcK$_{ATP}$, MitoK$_{ATP}$ and Na$^+$/K$^+$ ATPase antagonists on relaxation in intact and denuded rings**

The effects of voltage-dependent K$_v$, SarcK$_{ATP}$, MitoK$_{ATP}$ and Na$^+$/K$^+$-ATPase antagonists on lidocaine relaxation in intact rat aortic rings are shown in Fig. 4. After pre-contracted with NE, ring basal tensions were 3.3 ± 0.09 g; 3.5 ± 0.17 g; 3.4 ± 0.09 g; 3.4 ± 0.14 g (n = 8 each) for 4-AP, glibenclamide, 5-HD and ouabain groups, respectively; and not significantly different from NE with L-NAME and indomethacin controls (3.2 ± 0.19 g, n = 8). In endothelial intact aortic rings, exposure of rings to these antagonists did not alter lidocaine-induced relaxation compared to controls (Fig. 4).

In denuded rings, the effect of 1 mM 4-AP was to totally abolish relaxation up to 500 μM after which relaxation was 12 ± 5% (n = 8) compared to 39 ± 5% in denuded controls (i.e. 4-AP led to a 70% decrease in relaxation) (Fig. 5a). 4-AP inhibition was significant from 1 to 1000 μM lidocaine (p < 0.0001). The effect of glibenclamide (10 μM) had little or no effect on relaxation up to 10 μM lidocaine compared to denuded controls (Fig. 5b) and was ~20% lower at higher lidocaine concentrations; however, the differences were not significant. Exposure of denuded rings to 1 mM 5-HD led to ~50% decrease in lidocaine relaxation at 5 to 1000 μM lidocaine which was significant >50 μM (Fig. 5c). The presence of 100 μM ouabain, a Na$^+$/K$^+$-ATPase channel inhibitor, had little or no significant effect on lidocaine-induced relaxation (Fig. 5d).

**Effect of A$_{2a}$ and A$_{2b}$ antagonists in intact and denuded rat aortic rings**

The basal tension of NE-precontracted CSC group was 3.1 ± 0.16 g and PSB-0788 groups 3.7 ± 0.07 g (n = 8 each) and not significantly different from controls (3.2 ± 0.19 g, n = 8). Adenosine A$_{2a}$ antagonist 8-(3-chlorostyryl) caffeine (CSC) significantly decreased lidocaine relaxation in the intact rat aorta at 100 to 1000 μM (Fig. 6). Divergence began to occur at 50 μM lidocaine with relaxation values of 2, −2.8, −3.4 and 7.6% at 50, 100, 500 and 1000 μM lidocaine respectively. In direct contrast, the incubation with PSB-0788, an adenosine A$_{2b}$ antagonist, did not modify lidocaine-induced relaxation curve at any concentration used in NE pre-contracted aortic rings (Fig. 6).

In denuded rings, the basal tension of aortic rings with the presence of CSC (2.5 ± 0.15 g) or PSB-0788 (3.1 ± 0.18 g) was not significantly different. CSC had no effect on relaxation up to 10 μM lidocaine then strongly inhibited relaxation up to 500 μM (Fig. 6). The maximum lidocaine relaxation was 13 ± 6%, which was significantly lower than control denuded rings (39 ± 5%, p < 0.0001) (Fig. 6). The adenosine A$_{2b}$ receptor blocker PSB-0788 (10 μM) also decreased lidocaine relaxation by up to 50% but this effect was not significant (Fig. 6). At 10, 100, and 1000 μM lidocaine, the relaxation percentages were 7 ± 1, 10 ± 2, and 31 ± 3%, respectively compared to 16 ± 3, 19 ± 4, and 39 ± 5% in denuded controls (Fig. 6).

**Discussion**

Despite decades of investigation, the mechanisms of lidocaine relaxation in the rat thoracic aorta, and muscular resistance arterioles are not fully understood [12, 14–16, 20, 21]. We report in isolated rat thoracic rings, pre-contracted with NE, that lidocaine relaxation was: 1) biphasic from 1 to 10 μM and 10 to 1000 μM, 2) significantly enhanced by endothelial removal, particularly from 1 to 100 μM, 3) not significantly affected in the presence of L-NAME- and indomethacin in intact rings, 4) abolished by 4-AP in denuded rings and significantly reduced by 5-HD, and to a lesser extent glibenclamide, and 5) significantly reduced by A$_{2a}$ subtype antagonist from 100 to 1000 μM, but not A$_{2b}$. We discuss the possible physiological significance of the biphasic nature of lidocaine relaxation, enhancement after endothelial removal, and potential role for crosstalk with the A$_{2a}$ subtype and voltage-dependent K$_v$ and K$_{ATP}$ channels.

**Lidocaine relaxation was biphasic and endothelial dependent**

We found in oxygenated, glucose-containing Krebs Henseleit buffer, pH 7.4 at 37 °C: 1) little or no change in relaxation in rat aortic rings at low lidocaine concentrations, and 2) a strong endothelial dependence which enhanced relaxation after its removal (Fig. 2). The data
suggest that the presence of an intact endothelium acted like a “brake” to reduce lidocaine relaxation, and upon its removal activated some putative factor to enhance relaxation. Our findings are in contrast with those reported in rat cremaster skeletal muscle [14], epicardial porcine coronary arteries [50], human mammary arteries [52], and rabbit carotid arteries [15, 53], where lidocaine at low levels potentiated vasoconstriction, and at high concentrations led to relaxation.

Jembeck and Samuelson further reported in isolated rings from radial arteries that lidocaine led to significantly stronger contractions after the endothelium was mechanically removed [51]. Reasons for the differences are not clear at present but may relate to species, age, mode of sacrifice, physiological state, pre-contractile conditions activating different channels and receptors (e.g. alteration of the membrane smooth muscle potential with high K⁺ versus NE or phenylephrine to pre-contract isolated rings), tissue preparation, different endothelial removal procedures and possible damage, buffer conditions, temperature, P O₂ availability, and the sequence of drug additions and concentrations. Another important difference is vessel type; we studied the rat thoracic aorta, which is a large, highly elastic artery that normally offers little resistance to flow but assists in coupling the heart, as a pump and pressure-generator, to the arterial system by changing aortic compliance not resistance [54].

Fig. 4 Concentration-response curves to lidocaine with and without the presence of specific ion channel blockers in intact isolated rat aortic rings. a In the presence of 1 mM 4-aminopyridine. b In the presence of 1 mM 5-Hydroxydecanoate. c In the presence of 10 μM glibenclamide. d In the presence of 100 μM ouabain. Relaxation is expressed as percent of maximal relaxation to 100 μM papaverine. Points represent mean ± S.E.M of aortic rings in the presence of L-NAME and indomethacin. *P < 0.05 statistical difference in responses between the presence and the absence of inhibitors on intact rings. Lidocaine concentrations are on a log scale. Total animals n = 16.
That lidocaine relaxation occurred from 1 to 1000 uM is consistent with the study of Shan and colleagues who showed that lidocaine relaxed phenylephrine or KCl (60 mM) precontracted rat aortic rings in a concentration-dependent manner [13]. However, their study differed from ours because they showed lidocaine relaxation was not significantly modified by endothelium removal, and their aortic rings were obtained from rats sacrificed by stunning and cervical dislocation, not anesthesia [13]. Our study also agreed with Turan and colleagues who showed lidocaine relaxed phenylephrine-precontracted rabbit thoracic aorta intact and denuded rings, however, when lidocaine (1 to 100 uM) was applied 15 min before the addition of phenylephrine it produced contractions at high concentrations (up to 10 mM), and endothelium removal did not significantly affect contractile activity. This example demonstrates the dynamics of the pre-contractile state and the importance of specifying the sequence of drug administration, which can produce different results. Further studies are required to investigate these discrepancies in the thoracic aorta of rat and other species prepared from different modes of sacrifice, different precontracted states and basal tone.

Lidocaine relaxation enhancement involves an endothelium-smooth muscle coupling and possible activation of $K_{v}$ and $K_{ATP}$ channels
Since lidocaine relaxation displayed a strong endothelial-dependence (Fig. 2), it suggested a possible role for NO release or activation of the cyclo-oxygenase pathway and/or their interactions with the adrenoreceptors on vascular smooth muscle. Surprisingly, we found little or no effect of either L-NAME and indomethacin on lidocaine relaxation (Fig. 3) indicating that the putative relaxing factor after endothelial removal was neither NO nor prostacyclin. Other unknown factor(s) must be

**Fig. 5** Concentration-response curves to lidocaine with and without the presence of specific ion channel blockers in denuded isolated rat aortic rings. **a** In the presence of 1 mM 4-aminopyridine. **b** In the presence of 1 mM 5-Hydroxydecanoate. **c** In the presence of 10 uM glibenclamide. **d** In the presence of 100 uM ouabain. Relaxation is expressed as percent of maximal relaxation to 100 uM papaverine. Points represent mean ± S.E.M of aortic rings in the presence of L-NAME and indomethacin. *P < 0.05 statistical difference in responses between the presence and the absence of inhibitors on intact rings. Lidocaine concentrations are on a log scale. Total animals n = 16
released upon endothelial removal to enhance lidocaine relaxation. Another possibility is endothelial-dependent activation of smooth muscle voltage-dependent Kv channels and/or smooth muscle mitochondrial K\textsubscript{ATP} channels, since we showed that 4-AP completely abolished relaxation (Figs. 3a and 4a) and 5-HD led to ~50% inhibition in denuded rings (Figs. 4c and 5c).

Enhanced lidocaine relaxation may also have come from changing the cellular redox state and reactive oxygen species (ROS) derived from NAD(P)H oxidases [55, 56], as it has been reported that lidocaine at higher concentrations protects against ROS attack in rabbit abdominal aorta [9]. Rogers and colleagues further showed that 4-AP-sensitive Kv channels are redox sensitive and contribute to H\textsubscript{2}O\textsubscript{2}-induced coronary vasodilation [57]. In summary, we conclude that enhanced lidocaine relaxation after endothelial removal does not appear to involve the direct activation of NO or prostanoid-linked pathways, and that other relaxing factors and downstream signalling pathways, possibly involving Kv and/or 5-HD sensitive K\textsubscript{ATP} channels, are involved.

Smooth muscle adenosine A\textsubscript{2a} modulation may also be involved in the enhanced lidocaine relaxation

The present study also suggests an intriguing possibility for enhancing lidocaine relaxation may be activation of the A\textsubscript{2a} receptor on vascular smooth muscle. A surprising result was that lidocaine relaxation above 50 \(\mu\text{M}\) in intact and denuded rat aortic rings was significantly inhibited by 75 to 100% in the presence of A\textsubscript{2a} blocker 8-(3-chlorostyryl) caffeine (CSC) (Fig. 5). This implies that the A\textsubscript{2a} receptor may be involved in the presence or
absence of an intact endothelium. Assuming CSC has high specificity for A2a receptors [44], this antagonist may reduce lidocaine relaxation from one or more of the following mechanisms: 1) Directly or indirectly increasing Ca$^{2+}$ influx from extracellular sources such as L-type Ca$^{2+}$ channels [58], 2) increasing the release of Ca$^{2+}$ from intracellular stores (e.g. sarcoplasmic or endoplasmic) to increase cytosolic free Ca$^{2+}$, and/or 3) increasing myofibrillar contractile sensitivity to existing free Ca$^{2+}$ (signalled via the RhoA/Rho kinase pathway), increase cross-bridge cycling and development of force [55, 59]. Possible crosstalk between A2a receptors and lidocaine may also involve transmembrane domains of adenyl cyclase and other downstream signalling pathways to alter intracellular free Ca$^{2+}$ and/or myofibrillar sensitisation.

To our knowledge, little or no data exist on adenosine and lidocaine interactions in intact rat aortic rings or endothelial-vascular smooth muscle interactions. Adenosine A2a and A2b receptors are present on vascular endothelium and smooth muscle of many vessels [60, 61] and when activated can lead to vasodilation. A2a receptor vasodilation is thought to involve: 1) endothelial NO production which activates smooth muscle guanylyl cyclase via opening Kir channels [61], and/or 2) more direct smooth muscle A2a receptor activation which in turn stimulates mostly Gs proteins (and Gq) and cAMP signalling pathways to reduce intracellular Ca$^{2+}$ levels [61, 62]. In addition, adenosine A2a activation may activate sarcolemma Ca$^{2+}$ channels and regulate influx in large elastic arteries and resistance vessels. Stella and colleagues showed that activation of A2 receptors stimulates protein kinase A to inhibit L-type Ca$^{2+}$ channels in rod photoreceptors resulting in a decreased Ca$^{2+}$ influx [63]. Gubitz and colleagues have proposed dual A2a signalling involving the activation of both N- and P-type calcium channels by different G proteins and protein kinases in the some nerve terminals [64]. Gonvalves and colleagues showed that adenosine A2a receptors facilitated Ca$^{2+}$ uptake through class A calcium channels in rat hippocampal CA3 region [65].

Interestingly, Benkwitz and colleagues also showed that higher concentrations of lidocaine (1000 uM) in hamster oocytes potentiated Galphai-coupled A1 receptor signalling by reducing cyclic AMP production in a dose-dependent manner through an unidentified mechanism [29]. The authors proposed that lidocaine was not an A1-receptor agonist but enhanced adenosine-A1 receptor signalling. They argued that lidocaine interacted with a pool of already activated Gai present in the cytoplasm and thereby facilitated its ability to inhibit adenylate cyclase leading to lower cAMP [29]. We did not examine adenosine A1 receptor antagonism We conclude from our study that A2a receptor may have enhanced lidocaine relaxation activation by directly effecting vascular smooth muscle (Fig. 6), and this may have occurred by reducing intracellular Ca$^{2+}$ and/or myofibrillar contractile sensitization in intact isolated rat aortic rings, although the underlying mechanisms remain to be identified. Further studies are required to investigate the role of adenosine and lidocaine on membrane Ca$^{2+}$ channel modulation in isolated rat aortic rings.

Limitations of the study and possible physiological significance
The present study examined lidocaine relaxation in isolated rat thoracic rings using length-tension experiments and a number of antagonists of NO, prostanoids, K$\alpha$, K$_{ATP}$ and A2 receptors under normoxic and normal pH conditions from healthy rats. Before definitive conclusions can be drawn regarding the nature of unknown relaxation factor(s), it would be important to examine separately and in combination other drug antagonists and agonists of NO, prostanoids, K$\alpha$, Sarc- and Mito-K$_{ATP}$ channels and A2 receptors on lidocaine relaxation in intact and denuded rings. Furthermore, to gain greater mechanistic insight into the nature of voltage-dependent K$\alpha$ channels and lidocaine vasorelaxation electrophysiological experiments would be essential. Leukotrienes, and leukotriene synthase inhibitors, may also be of interest because they have been shown to modulate rat aortic ring relaxation [66]. Possible physiological significance of the present study relates to lidocaine’s effect to regulate in vivo compliance such as ventricular-arterial coupling functions linking the heart as a pump to tissue perfusion [67, 68]. However, further in vivo studies are required to test this hypothesis. Also our work may have clinical applicability on the ancillary properties of lidocaine at the site of injection during infiltration, nerve block, or epidural anesthesia [14], and on damaged endothelium such as in plaque formation, arterial and venous conduit protection for cardiopulmonary bypass grafting [69], prevention of vascular spasm during neurosurgery [70], lowering elevated intracranial pressure [71], lidocaine cardioplegia [72, 73], and other surgical applications [54].

Conclusions
We showed in isolated, oxygenated NE precontracted rat aortic rings that lidocaine relaxation was biphasic from 1 to 10 uM and 10 to 1000 uM. We further showed that lidocaine relaxation was significantly enhanced by endothelial removal, which did not appear to be NO or prostacyclin dependent. The putative unknown factor(s) responsible for enhanced relaxation may involve activation of smooth muscle voltage-sensitive K$\alpha$ and 5-HD sensitive channels or pathways, and possible crosstalk with A2a subtype receptor at higher lidocaine concentrations.
Abbreviations
4-AP: 4-aminopyridine; 5 HD: 5-Hydroxydecanoate; CSE: 8-(3-chlorostyryl)caffeine; MitokATP: Mitochondrial KATP channel; NE: Norepinephrine; NO: Nitric oxide; P5B-0788: 8-(4-(4-(4-chlorobenzyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine; SarckATP: Sarco/sarcoplasmic KATP Channel

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Availability of data and materials
The datasets supporting the conclusions of this article can be made available by emailing the authors.

Authors’ contributions
Both authors contributed equally to the design, implementation, literature and data analysis and the writing of the MS.

Competing interests
Arsyadi Arsyad and Geoffrey P. Dobson have no conflicts to declare.

Ethics approval and consent to participate
The animal study conforms to the Guide for Care and Use of Laboratory Animals (NIH, 8th Edition, 2011) and was approved by James Cook University’s Animal Ethics Committee, No. A1533.

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