

6.2±1.4CU respectively). L-NAME induced similar hindlimb vasoconstriction in all groups (~5CU) and attenuated the hypoxia-induced increase in IntFVC though this was only significant in N rats (9.7±1.5 vs 4.0±1.0CU) – suggesting a reduced role in CHU rats for NO in the hypoxic dilatation of skeletal muscle. SNP restored baseline IntFVC and allowed for a larger NO-dependent increase in IntFVC during hypoxia in both N and LCHU (9.7±1.8 & 7.8±1.1CU respectively) but not in ECHU (5.7±1.4CU). Protein analysis showed similar levels of eNOS in all groups in TA, but in SOL muscle eNOS was increased in ECHU and LCHU compared to N rats.

These results suggest that NO acts in a similar manner in both N and LCHU albeit to a lesser extent in the latter to produce an NO-dependent dilatation in hypoxia, further, it suggests this mechanism is not functional in ECHU. The fact that eNOS protein was unchanged (TA) or increased (SOL) suggests that the functional changes are not due to the lack of total eNOS protein, but more likely a reduction in NO bioavailability or eNOS function and this may have implications for the control of muscle blood flow under differing conditions.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C86

Hypoxia-induced impairment in rat respiratory muscles during development

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We studied the effects of hypoxia on contractile and endurance properties of respiratory muscles in the developing rat. Wistar rats aged postnatal day (P)19 and P29 were killed humanely and the diaphragm and sternohyoid (a representative pharyngeal dilator) muscles were surgically removed. Isometric contractile properties of isolated muscle strips were measured in tissue baths containing physiological salt solution at 30°C under hyperoxic (95%O₂/5%CO₂) or hypoxic (95%N₂/5%CO₂) conditions. Force-frequency relationship and fatigue index (*ie* ratio of force at 5min of fatigue to initial force) were examined. Fatigue was assessed in response to repeated tetanic contractions (40Hz, 300msec train duration) every 2 sec for 5 minutes. Hypoxia decreased specific force in the sternohyoid muscle but had no effect on diaphragm muscle force (peak force in sternohyoid muscle at P19 was 5.5±0.9 vs. 2.8±0.5*, P29 was 9.2±1.2 vs. 3.6±0.8*, mean±SEM N/cm², hyperoxia vs. hypoxia, P<0.05 ANOVA). We found that *in vitro* hypoxia significantly reduced muscle endurance in both the sternohyoid and diaphragm muscle. We also observed an age-dependent

decrease in endurance for both muscles in hyperoxic and hypoxic groups. Thus, sternohyoid fatigue index for P19 was (65.7±4.5% vs. 21.7±2.7%*), P29 was (37.4±2.7% vs. 2.7±1.7%*; mean±SEM hyperoxia vs. hypoxia, P<0.05 ANOVA). Diaphragm fatigue index for P19 was (80.7±2.7% vs. 27.9±2.7%*), P29 was (62±4.5% vs. 19.7±1.8%*, mean±SEM, hyperoxia vs. hypoxia, P<0.05 ANOVA). We conclude that hypoxia impairs respiratory muscle function. Our results suggest that the sternohyoid muscle is more vulnerable to hypoxic insult than the diaphragm muscle. We speculate that this is due to their different fibre type characteristics. The mechanism for hypoxia-induced muscle impairment remains unknown. However, the effects of hypoxia may have implications for the control of airway patency *in vivo*.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

C87

The interaction of stretch, intraluminal pressure and *E.coli* heat stable (STa) enterotoxin on jejunal fluid absorption in the anaesthetised rat

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A perfused intestinal loop preparation was used to measure luminal uptake of fluid *in vivo* by means of fluid volume recovery from the jejunum of the anaesthetised rat (70 mg/Kg i.p. sagatal). All procedures were carried out in conformity with current UK legislation. Data is given as the mean plus the standard error with the number of experiments in brackets. Significance was tested by 't'-test. Distension of the jejunum with a polythene loop in the lumen reduced fluid absorption (p<0.001) from 116±18 (7) ul/cm/hr to 40±5 (10) ul/cm/hr. Heat stable (STa) toxin from *E.coli* reduced fluid absorption further to 14±13 (6) ul/cm/hr in the stretched intestine, not significantly different from zero fluid absorption. Distension by 30 cm hydrostatic pressure reduced fluid absorption (p<0.01) to 52±10 (6) ul/cm/hr. Combination with STa reduced fluid absorption to 29±10 (5). Lack of net secretion implies that distension does not initiate a secretory event but prevents absorption. The lack of super-imposition of STa and distension effects implies a common absorption mechanism inhibited by both.

Low rates of fluid absorption by coil distension were not restored by serosal application of lidocaine, *i.v.* hexamethonium or luminal perfusion of atropine. In contrast, luminal atropine did restore fluid absorption in jejunum distended by hydrostatic pressure, from 52±10 (6) ul/cm/hr to 103±15 (5) ul/cm/hr, not significantly different from the undistended jejunal value. The neural component to the inhibition of absorption is likely to be mediated through an axon reflex within a cholinergic neuron. In contrast, neither *i.v.* hexamethonium, serosal lidocaine nor luminal atropine restored fluid absorption after exposure

showed a strong relationship between HB_{insp} and changes in IE/II ratio ($r = 0.85$, $p < 0.01$) but not with changes in RSA magnitude ($r = 0.19$, $p = 0.55$).

These results suggest that, contrary to the common view, RSA magnitude does not cause significant heartbeat clustering into inspiration in humans. The mechanism behind associations between RSA and indices of gas exchange efficiency and the underlying function of RSA remain unclear.

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PC121

Virally-mediated expression of ATP degrading enzymes as a new tool to study ATP-mediated signalling *in vivo* and *in vitro*

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Recent data suggest that ATP is released in the medulla in response to hypoxia and hypercapnia and may mediate excitation of presympathetic rostral ventro-lateral medulla (RVLM) neurones. Thus, during hypoxia or hypercapnia ATP is released within the areas where these neurones are located (Gourine et al. 2005b). Activation of P2 receptors in the RVLM evokes marked increases in blood pressure and renal sympathetic nerve activity (Horiuchi et al. 1999; Ralevic et al. 1999; Thomas et al. 2001) while exogenous ATP excites bulbospinal presympathetic RVLM neurones (Ralevic et al., 1999). However, further studies into the role of ATP in central respiratory and sympathetic chemosensitivity are hampered by the lack of selective pharmacological tools. The existing P2 antagonists (e.g. PPADS and others) are notorious for their lack of specificity and are not suitable for chronic *in vivo* studies. Here we validate a novel strategy to study the role of ATP-mediated signalling in the CNS based on a viral gene transfer of ATP-degrading enzymes. Extracellular ATP is broken down in several steps and this is catalyzed by ectonucleoside triphosphate diphosphohydrolases. Another family of enzymes, the alkaline phosphatases convert a variety of substrates, including ATP to adenosine. Here we have successfully used one such enzyme, the placental alkaline phosphatase (PLAP, the human gene) in a proof-of-principle experiment using a lentiviral vector LVV-EL1 α -hPLAP. This LVV

expresses PLAP under control of a non-specific EL1 α promoter leading to the gene expression in neurones, glia and other cells. In rats ($n=8$) four unilateral injections of LVV-EL1 α -hPLAP were made unilaterally into the right RVLM (under ketamine (60 mg/kg) and medetomidine (250 μ g/kg) i.m. anaesthesia). Seven days later horizontal slices containing the ventral medullary surface were prepared and used for an *in vitro* experiment as described in (Gourine et al. 2005a; Gourine et al. 2005b). ATP microelectrode biosensors were used to determine release of ATP from the ventral surface chemosensitive areas in response to isohydric hypercapnia using aCSF solution in which $NaHCO_3$ was increased to 50 mM (isototically replacing NaCl) and equilibrated with 10% $CO_2/90\% O_2$ (pH ~ 7.45 , $pCO_2 \sim 65$ mmHg at $37^\circ C$). It was found that the amount of ATP released in response to isohydric hypercapnia was significantly (by $\sim 50\%$; $P=0.008$) smaller on the transduced side of the medulla. Thus, expression of PLAP in the RVLM can be used as a highly effective approach for rapid degradation of ATP released into the extracellular space during chemosensory stimulation. We conclude that virally-mediated expression of ATP degrading ecto-enzymes can be used as a novel tool to study the multiple functional roles of ATP-mediated signalling in the central nervous system.

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PC122

Effects of chronic hypoxia on diaphragm function in juvenile and adult rats

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Chronic hypoxia occurs in normal individuals at altitude and also in patients suffering from respiratory disease. Skeletal muscle structure and oxidative capacity are age dependent are known to be affected by chronic hypoxia. The aim of this study was to examine the age-dependent effects of chronic hypoxia on diaphragm muscle contractile and endurance properties. Adult (12 week old) and juvenile (3 week old) Wistar rats were exposed to either hypobaric hypoxia (barometric pressure 380mmHg) ($n=12$) or normobaric normoxia ($n=12$) for 6 weeks. At the end of the treatment periods, isometric contractile and endurance properties of isolated strips of diaphragm muscle were measured in tissue baths under hyperoxic

(95%O₂/5%CO₂) conditions maintained at 30°C. Force-frequency relationship and fatigue tolerance was examined. For the force-frequency relationship, the diaphragm was stimulated at 10, 20, 30, 40, 60, 80 and 100 Hz (300ms train duration) allowing a 2-min interval between stimulation. Five minutes following the force-frequency protocol, fatigue was assessed in response to repeated tetanic contractions (40Hz, 300ms) every 2 seconds for 5 minutes.

Specific force was reduced and fatigue resistance significantly increased in both adult and juvenile rats exposed to chronic hypoxia. The fatigue index (ie ratio of force at 5 min of fatigue to initial force) was significantly altered by chronic hypoxia in adult rats (32.5±4.8 vs. 51.7±3.2%; % of initial force, control vs. hypoxia*, P<0.05, ANOVA). This effect was similar in juvenile rats 43.0±1.9 vs. 67.4±3.3, % of initial force, control vs. hypoxia*, P<0.05, ANOVA).

In this study, chronic hypoxia increased endurance in both adult and juvenile rats. This effect could be indicative of a fibre-type transition toward a more oxidative phenotype due to hypoxia or hypoxia-induced hyperventilation. Fatigue resistance was more pronounced in the juvenile compared to adult rats for chronic hypoxia groups. This study has shown developmental and hypoxia-induced plasticity in the rat respiratory muscle.

Health Research Board in Ireland

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PC123

Profound effects of AA and its metabolites on HPV of rat intrapulmonary artery

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Hypoxic pulmonary vasoconstriction (HPV) is the rapid, reversible increase in pulmonary vascular resistance that occurs when the alveolar oxygen tension falls below a threshold level. HPV demonstrates a transient constriction (phase I) superimposed on a sustained constriction (phase II). An interval of 60 minutes between repeated hypoxic mediums was employed in our protocol to determine the regulation mechanism of HPV. Consistent to previous studies, verapamil at the concentration of 20 µM, which completely abolished 80 mM [K⁺]_o induced vasoconstriction, only attenuated phase I by 19.8±4.5 % (n= 6); whereas SKF 96365 (40 µM) significantly decreased phase I by 31.8 ± 5.6 % (n=6) and 2APB (50 µM) significantly reduced phase I by 18.1 ± 6.8 % (n=7) and phase II by 50.1 ± 7.6% (n=7). These results support that Ca²⁺ entry via voltage gated channel (L-type) and non-voltage gated channel e.g. TRP channel mediates HPV. In a previous work, we have demonstrated that arachidonic acid (AA) could inhibit hypoxia-induced [Ca²⁺]_i elevation in primary cultured human pulmonary artery smooth muscle cells. However, in rat intrapulmonary artery, AA alone at concentration 10 µM (n=8) induced a significant transient vasoconstriction, equivalent to 29.1 ± 9.8% of vasoconstriction induced by 80 mM [K⁺]_o. AA (10 µM) significantly

attenuated the phase II (c) of HPV by 25.5 ± 4.5 % (n=7) and had no effect on phase I of HPV. The inhibitory effect of AA was mediated by downstream metabolites of AA via cyclooxygenase (COX). ETYA is a pharmacology analogue of AA and also inhibits COX, LOX and CYP450 at distinct dosage ranges. ETYA at concentration of 20 µM, which could inhibit COX, significantly inhibited phase I by 59.5 ± 6.5 % and phase II 35.9 ± 7.6 % of HPV (n=9). Whereas ETYA at concentration of 1 µM, which could inhibit LOX, had no effect on either phase I or II of HPV (n=6). Blockage of COX by DuP697 reduced the phase II of HPV by 27.6 ± 6.8 % (n=6) and it was consistent to the effect of AA. Taken together, the data suggested that metabolites of AA via COX involve in mediating phase II of HPV, which is endothelium dependent. Other metabolites in addition to products of COX may participate in mediating phase I of HPV. Blockage of CYP450 by SKF525 significantly attenuated the phase I of HPV by 24.9 ± 7.5 % (n=6). EET (200 nM), a metabolite of AA via CYP450 epoxygenase, reduced the phase I of HPV by 10.5 ± 5.6 % (n=5), suggesting the vasoconstriction might be derived from effects of the metabolites via CYP450 hydroxylase, but not epoxygenase. In addition, either application of PGI₂ (n=6) or inhibition of PGI generation by 15 HPETE (n=6) had no significant effects on HPV, implicating HPV in rat intrapulmonary artery is primarily determined by increase of vasoconstrictors and not by reduction of vasodilators.

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PC124

Bronchial hyperresponsiveness and nitric oxide measurement in exhaled air of asthma patients as markers of airways inflammation

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Aim of the study: The purpose was to investigate the correlations between bronchial hyperresponsiveness and nitric oxide (NO) levels measured in exhaled air and to establish their involvement in airways inflammation process in allergic patients.

Material and methods: 188 subjects (mean age 28 ± 7.5; 47.1% male, 52.9% female) with chronic cough were investigated under the suspicion of bronchial asthma/allergic rhinitis. After signing the written informed consent and based on questionnaire methods (ACT for asthma, and SQUAR-E for allergic rhinitis), in accordance with the international criteria (GINA for asthma, and ARIA for allergic rhinitis), 145 subjects were included into study as allergic patients with mild and moderate asthma ± allergic rhinitis, without corticotherapy for at least 2 months. Depending on pulmonary function disorders, the bronchomotricity tests are performed, including the bronchial

methacholine provocation test, in patients having FEV1 (forced expiratory volume in 1 second) $\geq 60\%$ of ideal value or distal obstructive syndrome. Methacholine solution is inhaled using Wright nebulizer, in progressively increasing doses, starting from 0.075 mg/ml to the 15 mg/ml. Bronchial hyperreactivity was assessed by PC20 value (the methacholine concentration which induces a 20% decrease of FEV1), which also indicates the severity of bronchial hyperreactivity. Nitric oxide level in the exhaled air (FENO) was measured using the computer NOXIMETER device, which determines this concentration every 2 minutes of forced respiration.

Results: Asthma severity, according to GINA criteria was established in 59 allergic patients after bronchial provocation tests, as mild persistent asthma (41), moderate asthma (17), and severe asthma (1). The mean severity degree of bronchial hyperreactivity in methacholine testing, estimated by PC20 was 3.26 ± 2.6 mg/ml. The ACT score at the beginning of the study was 14.37 ± 2.4 . NO from exhaled air was correlated (significant correlation index $r = 0.51$) with the degree of bronchial hyperreactivity, and FENO was determined between 5 and 173 ppb. Conclusions: For assessment of bronchial inflammation, the bronchial methacholine provocation test (indirect marker of inflammation) is significantly correlated with NO levels (direct marker of inflammation) from exhaled air, but separately, none of these tests has significant specificity and sensibility, which results in more complex approaches that are required for diagnosis.

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Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PC125

Cytokine expression in the diaphragm in response to acute resistive loading: Regulation by Nitric oxide and oxidative stress

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Introduction:

Strenuous diaphragmatic contraction associated with loaded breathing induces an inflammatory response observed both within the diaphragm (1) and systemically (2). ROS production increases (3) whereas NO production decreases (4) in the diaphragm in response to acute resistive breathing.

Methods:

Anesthetized (ketamine 100mg/kg ip), tracheostomized, spontaneously breathing female adult wistar rats were subjected

for 6 hours to moderate inspiratory resistive loading (50% of the maximum inspiratory pressure) (1, 4). Based on the IRL protocol two independent set of experiments performed: 1) rats undergoing IRL with the pre-treatment (RB-LN) or not (RB) of the non-selective NOS inhibitor L-NAME (100mg/kg/3h), 2) IRL rats treated with: a) vitamin C (RB-vC), 500mg/kg, b) vitamin A (RB-vA), 195mg/kg, c) N-Acetyl-L-cysteine (RB-NAC), 1gr/kg, d) oxypurinole (RB-oxp), 50mg/kg. All drugs were administered ip 30 min before the initiation of IRL (for oxp an additional treatment the day before, 50mg/kg/12h, was performed. Immediately after the 6h IRL the diaphragm was excised and cytokine protein expression was analysed with ELISA (RnD Systems). Sham-operated rats pretreated with either saline or the respective drugs were studied as control (Ctr). All statistical correlations were performed by Mann-Whitney analysis.

Results

The cumulative ELISA results are presented in Table 1.

Blockage of NO production with L-NAME administration resulted in augmentation of the IRL-induced cytokines production within the diaphragm (RB-LN vs RB, $p < 0.05$ and RB vs Ctr, $p < 0.05$).

NAC pre-treatment of the rats undergoing IRL, caused a statistically significant intra-diaphragmatic reduction of IL-6, TNF- α and IL-1b (for all correlations $p < 0.05$) whereas, vitamin A administration resulted in a similar reduction of IL-1b and a borderline decrease in TNF- α ($p = 0.056$). Furthermore, oxypurinol treatment reduced only TNF- α production in the diaphragm of both control ($p < 0.05$) and RB animals ($p < 0.05$). In contrast, administration of vitamin C did not affect cytokine production at all.

Conclusions

1) Inhibition of endogenous NO synthesis resulted in significant elevation of cytokines (IL-6, IL-1b, IL-10, TNF- α) expression in the diaphragm of rats exposed to moderate inspiratory resistive loading.

2) Antioxidant treatment modifies intra-diaphragmatic resistive breathing induced cytokine expression.

TABLE 1

Experimental Protocol 1 [values in pg/mg of total protein- data shown as mean \pm SEM (n)]				
	IL-6	IL-1b	IL-10	THF- α
Ctr	46.9 \pm 1.9 (12)	57.5 \pm 2.8 (12)	22.1 \pm 1.7 (12)	19.1 \pm 1.0 (12)
Ctr-LN	46.4 \pm 2.5 (10)	58.3 \pm 3.5 (10)	23.5 \pm 2.4 (10)	20.4 \pm 1.8 (10)
RB	64.3 \pm 5.2 (15)	76.9 \pm 5.8 (15)	34.4 \pm 3.8 (15)	33.5 \pm 2.9 (15)
RB-LN	102.1 \pm 12.8 (15)	103.9 \pm 11.6 (15)	53.1 \pm 5.7 (15)	51.3 \pm 4.4 (15)

Experimental protocol 2 [values in pg/mg of total protein- data shown as mean \pm SEM (n)]			
	IL-6	IL-1b	THF- α
Ctr	14.5 \pm 1.3 (9)	22.9 \pm 1.9 (9)	31.1 \pm 2.5 (9)
Ctr-vC	13.4 \pm 1.9 (6)	29.4 \pm 4.1 (6)	30.5 \pm 3.4 (6)
Ctr-vA	19.6 \pm 3.1 (6)	24.9 \pm 2.5 (6)	33.9 \pm 0.3 (6)
Ctr-IAC	17.5 \pm 2.5 (6)	30.7 \pm 3.4 (6)	29.5 \pm 0.9 (6)
Ctr-oxp	14.4 \pm 2.0 (6)	62.3 \pm 9.8 (6)	20.1 \pm 2.9 (6)
RB	46.5 \pm 5.8 (8)	40.0 \pm 3.1 (8)	55.9 \pm 6.9 (8)
RB-vC	35.8 \pm 2.8 (9)	36.3 \pm 2.1 (9)	48.6 \pm 4.6 (9)
RB-vA	50.3 \pm 6.0 (10)	23.5 \pm 2.3 (10)	40.3 \pm 2.8 (10)
RB-IAC	22.7 \pm 1.6 (7)	23.0 \pm 3.0 (7)	32.4 \pm 3.0 (7)
RB-oxp	32.3 \pm 4.6 (7)	51.1 \pm 10.3 (7)	26.6 \pm 5.7 (7)

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PC126

Tempol, a SOD-mimetic, improves muscle function in a rat model of sleep apnoea

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Obstructive sleep apnoea (OSA) is a common disorder characterised by repeated occlusions of the upper airway during sleep. Upper airway muscle dysfunction is implicated in the pathophysiology of OSA. We have shown that intermittent hypoxia – a feature of OSA due to recurrent apnoea – impairs respiratory muscle function. In this study, we tested the hypothesis that antioxidant treatment following chronic IH exposure would improve muscle function in our rat model of OSA.

15 adult male Wistar rats were placed in chambers and exposed to alternating periods (90s) of normoxia and hypoxia. Control rats (n=15) were placed in identical chambers and were exposed to an air/air cycle continuously under identical conditions in paired studies. Exposures lasted 8 hours per day for 9 days. Following the treatments, animals were killed humanely. The paired sternohyoid muscles were dissected out and prepared for in vitro examination. Isometric contractile properties of isolated strips of sternohyoid muscle were examined in tissue baths under hyperoxic (95%O₂/5%CO₂) or hypoxic (95%N₂/5%CO₂) conditions in the absence (control) or presence of the superoxide dismutase (SOD) mimetic, Tempol (10mM). Specific force was measured in response to stimulus frequencies ranging from 10-100Hz.

Under in vitro hyperoxic conditions, IH caused a reduction in force at 60 to 100Hz [Peak tetanic force at 100Hz was 22.7±0.8 vs. 15.9±0.9* N/cm², control (n=8) vs. IH (n=8), *P<0.001 ANOVA]. Tempol had a positive inotropic effect at high stimulus frequencies in both normoxic and IH-treated rats [Force at 100Hz in hyperoxia was 26.4±1.0 and 22.8±1.4 N/cm², control+tempol (n=7) and IH+tempol (n=7); both significantly different from their respective controls, ANOVA. The relative increase in force was greater in the IH-treated rats compared to normoxia. Under in vitro hypoxic conditions, forces were significantly lower than hyperoxic values but there was no difference between normoxia and IH-treated rats.

This study illustrates that chronic IH decreases force production of rat sternohyoid muscle but has no effect on force generation during in vitro hypoxia. Furthermore, Tempol has a positive inotropic effect on sternohyoid muscle in both groups, but the relative increase in force was greater in IH-treated animals with a recovery of force to control values. We conclude that chronic IH causes maladaptive plasticity in a pharyngeal dilator muscle and as such may be implicated in the pathophysiology of OSA. We conclude that antioxidants may be beneficial as adjunct therapies in the treatment of OSA.

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PC127

Patterns of Ca²⁺ signals induced by purinergic and adrenergic stimulation of smooth muscle cells from rat renal resistance arteries

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Renal vascular tone, which determines renal blood flow, is controlled by sympathetic nerves via activation of α_1 -adrenoceptors (α_1 -ARs) and P2X purinoceptors (P2X-Rs) [1]. Activation of these causes a robust increase of [Ca²⁺]_i. The aim of this work was to visualise with high temporal resolution sub-cellular calcium events evoked by stimulation of α_1 -ARs or P2X-Rs in renal vascular smooth muscle cells (RVSMCs) and to analyse the dynamics of these events. Experiments were performed on RVSMCs freshly isolated from rat renal resistance arteries, which were separated from the kidney using an iron oxide sieving technique [2]. Analysis of gene expression in single RVSMCs (250 cells were collected with a glass micropipette) using RT-PCR technique confirmed expression of the genes encoding for α_{1A} -ARs and P2X₁-Rs, but not for α_{1B} -ARs, P2X₃-Rs and P2X₅-Rs. Changes of [Ca²⁺]_i induced by stimulation of α_1 -ARs or P2X-Rs in RVSMCs loaded with the Ca²⁺ indicator Fluo-4AM were visualised using fast (33-40 Hz) x-y confocal imaging. Data are presented as mean ± S.E.M.; independent Student's t-test was performed to verify statistical significance of the results. Stimulation of α_1 -ARs with 10 μ M noradrenaline (NA) or P2X-Rs with 10 μ M α,β -methylene adenosine 5'-triphosphate (AMP-CPP) evoked a rise in [Ca²⁺]_i, which consisted of transient and sustained components and was initiated by a sub-plasmalemmal [Ca²⁺]_i upstroke (SPCU) [3]. AMP-CPP-evoked SPCUs had a significantly (p<0.0001) faster upstroke (mean time-to-peak 0.72±0.06 s; n=13) than NA-induced ones (mean time-to-peak 1.56±0.15 s; n=12). Time-to-peak of the AMP-CPP-induced global [Ca²⁺]_i rise (0.79±0.07 s; n=19) was also significantly (p<0.0001) shorter than the NA-induced one (1.89±0.18 s; n=17). Both AMP-CPP- and NA-induced [Ca²⁺]_i transients, however, were of similar duration: the full duration at half-maximal amplitude of the transients were 4.08±0.30 s (n=19) and 4.49±0.35 s (n=17), respectively. Simultaneous imaging of AMP-CPP-induced [Ca²⁺]_i changes in cytosol and mitochondria ([Ca²⁺]_m) using Fluo-4 and Rhod-2, respectively, revealed that [Ca²⁺]_m started to rise with a delay of 0.34±0.08 s following initiation of the [Ca²⁺]_i transient and reached its peak 1.02±0.17 s later (n=14). Mitochondrial sequestration of Rhod-2 was confirmed by double staining with MitoTracker Green FM (MTG; a