C19

The role of muscarinic receptors in the control of ovine submandibular function

G. Tobin and A.V. Edwards

* *Department of Pharmacology, University of Goteborg, Medicinaregatan 15D, Goteborg 413 90, Sweden and* †*Physiological Laboratory, University of Cambridge, Downing Street, Cambridge, CB2 3EG, UK*

This study was undertaken to determine which muscarinic receptors mediate parasympathetic responses in the ovine submandibular gland. The effects of selective muscarinic blocking agents on secretory and vascular responses to electrical stimulation of the chorda-lingual nerve were determined and morphological correlates were assessed by Western blotting. Anaesthesia was induced and maintained with sodium pentobarbitone (Sagatal; 15–30 mg kg^{-1} I.V., then 0.1–0.3 mg min^{-1} kg^{-1} I.V.). The ipsilateral ascending cervical sympathetic nerve was cut to eliminate effects of sympathetic activity and the animals were eventually killed with a lethal dose of barbiturate.

Stimulation of the cut end of the parasympathetic chorda-lingual nerve continuously at 2 Hz, or in bursts of 1 s at 20 Hz every 10 s, for 10 min induced similar fluid responses (19 ± 5 *vs.* 21 ± 5 μl min^{-1} g $gland^{-1}$; $n = 10$) although the protein output was about 50 % greater ($P = 0.015$; Student's t test) during stimulation in bursts. The vasodilator response was also significantly greater at 20 Hz 1:10 than at 2 Hz continuously as reflected by the fall in submandibular vascular resistance -53 ± 5 *vs.* -45 ± 4 %; $P = 0.016$; $n = 10$). Stimulation at 8 Hz induced a fluid response approximately three times as large (74 ± 11 μl/min g gland; $n = 10$) and a protein output 5 times greater than at 2 Hz; the reduction in vascular resistance was -75 ± 3 % ($n = 10$). Pirenzepine (40 μg/kg I.V.) significantly reduced the fluid response at all frequencies tested ($P < 0.05$–0.01), most conspicuously at 2 Hz where it was reduced by 85 %, but did not cause any significant change in the protein output or the vascular response. *p*-Fluoro-hexahydro-sila-diphenidol (4 μg/kg I.V.) in the presence of pirenzepine tended to further reduce the salivary secretion (reduced by 20 % at 8 Hz; n.s.), without causing any further change in the vascular or protein responses. Methoctramine (100 μg/kg IV), had no effect on either the fluid or the vascular response but significantly increased the protein output during the initial few min (2–4) of stimulation at 2 (+170 %; $P < 0.007$) and 20 Hz 1:10 (+125 %; $P = 0.014$), but not at 8 Hz. Western blot studies employing muscarinic receptor subtype-specific antibodies, revealed distinct bands corresponding to muscarinic M1, M3 and M5 receptors, with a faint band corresponding to the M4 type.

Thus, M1 receptors occur in the ovine submandibular gland and contribute to the secretory response to parasympathetic stimulation but have little effect on the vasodilatation. Inhibitory muscarinic receptors seem to be of the M4 subtype, and blockade by methoctramine of neuronal inhibitory receptors causing increases in transmitter release would explain the increase in protein output.

All procedures accord with current UK legislation

C20

A new biosensor for the measurement of extracellular ATP in the CNS

E. Llaudet, M. Droniou and N. Dale

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

ATP is an important neurotransmitter and modulator that can act at two classes of receptors within the CNS. As many of the functions of ATP are incompletely understood, the ability to measure ATP release from the CNS in real time is a valuable tool to further understand its functions. Here we present a micro-biosensor capable of rapid *in situ* detection of ATP in neuronal tissue.

Building on our previous work (Llaudet *et al.* 2003) we have entrapped 2 enzymes, glycerol kinase and glycerol-3-phosphate oxidase, in a thin robust layer around a Pt microelectrode. The system is based on the amperometric detection of peroxide produced by the oxidation of glycerol-3-phosphate; in the first step of the enzyme cascade glycerol kinase catalyses the transfer of a phosphate from ATP to glycerol, which therefore needs to be present at concentrations of at least 500 μM. The modified electrode shows impressive selectivity, sensitivity (150 mA/M/cm_2) and speed of response (10 s 10–90 % signal) towards ATP. The sensor is independent of glycerol concentrations above 500 μM and of oxygen, but while the magnitude of the response to ATP is independent of pH, over the range pH 6–8, the base line current shows a slight pH dependence. The electrode size can range from 25 to 100 μm in diameter and 0.5 to 2 mm in length which allows them to be inserted into most tissues without causing significant damage.

We have tested this new biosensor by measuring the release of ATP from the spinal cord of *Xenopus* embryos during swimming. *Xenopus* embryos were prepared under MS222 anaesthesia for extracellular ventral root recordings using previously described methods (Dale, 1995). The sensor was aligned with the ventral part of the spinal cord and simultaneous ventral root recordings made. We detected transient ATP release throughout the swimming episode that was phase-locked to ventral root discharge on each motor cycle. With the aid of this new biosensor we have for the first time provided direct evidence for the rhythmic release of ATP during spinal cord activity.

Dale N (1995). *J Physiol* **489**, 489–510.

Llaudet E *et al.* (2003). *Biosens Bioelectron* **18**, 43–52.

All procedures accord with current UK legislation

C23

Cardiovascular and respiratory regulation through brainstem adenosine receptors in acute ethanol microinjection into NTS

I. Rocha*, G. Postolache† and L.S. Carvalho*

* *Instituto de Fisiologia, Faculdade de Medicina de Lisboa, Lisbon, Portugal and* † *Faculty of Biology, Al.I.Cuza University, Iasi, Romania*

Previous studies have suggested that adenosine may be an important mediator of ethanol effects in the brain. In the present study, the cardiovascular and respiratory effects of acute ethanol microinjection into nucleus tractus solitarius (NTS), before and after dipropilsulfophenilxanthine (DPSPX), an adenosine antagonist, were investigated.

Experiments were performed in male Wistar rats ($n = 7$), anaesthetized (α chloralose, 100 mg/kg), treated with a neuromuscular blocker (pancuronium, 4 mg/kg/h) and artificially ventilated. The depth of the anaesthesia was maintain by ensuring the absence of a withdrawal reflex before paralysing the animal and changes on arterial blood pressure and heart rate to pinching a paw after the administration of a muscle blocker. Arterial blood pressure (BP), electrocardiogram (ECG), phrenic nerve activity and heart rate were monitored. A craniotomy was carried out to allow the insertion of multi-barrelled microelectrodes for electrical stimulation (50 Hz, 1 ms, 20–40 mA), and for unilateral microinjections of ethanol (50 mM, 50 nl) and DPSPX (0.1 mM, 50 nl) into NTS. The baroreflex function was evaluated by peripheral injection of phenylephrine (4 μg/kg) in basal conditions and after microinjection of ethanol into the NTS. The slope of the regression line between beat-to-beat values of RR-interval plotted against the systolic blood pressure values of the preceding cardiac cycles [RRI(i+1) *vs.* SAP(i)] was considered to be index of baroreflex sensitivity. All the experimental procedures were performed according to Portuguese and EU laws on animal research. At the end of the experiments, animals were humanely killed.

Our results show a decrease of mean blood pressure (29.58 ± 4.9 mmHg, paired t test $P < 0.0001$) and heart rate (30.833 ± 9.6 bpm, $P < 0.01$, t test) accompanied by a decrease in nerve phrenic activity, both in frequency and amplitude (duration of response from 1 to 2 min) as result of ethanol microinjection in the NTS. This was accompanied by a markedly decreased of baroreflex slope (from 0.189 ± 0.038 to 0.1346 ± 0.031 ms/mmHg, $P < 0.05$, t test). Following the treatment with DPSPX, the effects of ethanol microinjection on BP, HR and phrenic nerve activity are greatly attenuated and to a lesser extent baroreflex function.

In conclusion, hypotension, bradycardia and respiratory depression produced by acute ethanol microinjection are at least in part mediated by adenosine receptors. The significantly change of baroreceptor reflex sensitivity observed on ethanol microinjection into NTS underlines centrally neurons implication on reported decreased baroreflex function to peripherally alcohol administration

All procedures accord with current national and local guidelines

C24

Epoxygenase activity in astrocytes is important for maintenance but not initiation of activity-related dilatation of cerebral arterioles in brain slices

T.A. Lovick and B.J. Key *

*Departments of Physiology and *Pharmacology, University of Birmingham, Birmingham B15 2TT, UK*

Increases in neuronal activity are accompanied by local increases in cerebral blood flow that facilitate increased delivery of nutrients to meet the needs of the active neuropil. It has been suggested that astrocytes act as an intermediary stage in the process of flow-metabolism coupling by releasing vasodilator products of arachidonic acid metabolism from their perivascular end-feet (Harder *et al.* 2000).

In the present study we have investigated the dynamics of this process in cortical arterioles maintained *in situ* in brain slices. Coronal slices, 300–350 μm thick were prepared from brains of urethane-anaesthetised (1.5 g kg^{-1} ip) male rats, 130–230 g body weight and maintained in artificial cerebrospinal fluid (ACSF) at 33°C. Images of parietal cortical arterioles (internal diameter (ID) 8.5–16.5 μm) were captured using a CCD camera and Openlab image analysis software (Improvision Ltd) as described previously (Lovick *et al.* 1999).

In 7 vessels inclusion of 75 nM U46619 in the ACSF decreased internal diameter -12.8 ± 2.5 %, mean ± S.E.M.) and induced rhythmic contractions (9.9 ± 1.4 min^{-1}) of smooth muscle cells in the vessel wall (vasomotion). In these pre-constricted vessels, addition of AMPA (1 μM for 30 min) produced a 12.1 ± 1.5 % increase in ID ($P < 0.001$, Student's unpaired t test) and the frequency of vasomotion decreased by 8.0 ± 1.4 contractions min^{-1} ($P < 0.001$). The response was maintained without decrement throughout the superfusion period and on washout, returned to control values within 20 min. In a further 7 vessels the response to AMPA was tested in the presence of the epoxygenase inhibitor miconazole (20 μM). Addition of miconazole to resting vessels (no U46619 in ACSF) produced a 11.1 ± 2.7 % decrease in ID and vasomotion increased to 9.9 ± 2.3 min^{-1}. These values are comparable to the pre-constriction produced by U46619. Addition of AMPA to miconazole-treated vessels produced an initial dilatation (9.8 ± 3.1 % increase in ID, $P < 0.003$) and reduction in vasomotion -4.6 ± 1.5 min^{-1}, $P < 0.02$). However, the response was not maintained. Within 10 min the vessels began to constrict, vasomotion increased and by the end of the 30 min AMPA superfusion period the values had returned to control levels.

The results suggest that epoxygenase activity is not a pre-requisite for initiating activity-related dilatation in cortical arterioles even though it is clearly important for maintaining the response. Any contribution of epoxygenase products to the early phase of activity-related dilatation is likely to be due to release from pre-formed membrane stores.

Harder DR *et al.* (2000). *Acta Physiol Scand* **168**, 543–549.

Lovick TA *et al.* (1999). *Neuroscience* **92**, 47–60.

This work was supported by the British Heart Foundation

All procedures accord with current UK legislation

C25

Evidence that vagal bradycardias evoked by baroreceptor and chemoreceptor afferents involve the activation of central 5-HT$_7$ receptors

Daniel O. Kellett, Andrew G. Ramage and David Jordan

Departments of Physiology & Pharmacology, UCL, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK*

Central 5-HT$_{1A}$ receptors play an important role in the control of vagal bradycardias evoked by vagal afferents (Xth nerve) but not chemoreceptor afferents (IXth nerve; Skinner *et al.* 2002). Recently 5-HT$_7$ receptors have also been implicated in the control the vagal bradycardias evoked by vagal (cardiopulmonary) afferents (Kellett *et al.* 2003) using the selective 5-HT$_7$ receptor antagonist SB-269970 (Hagan *et al.* 2000). The present experiments have investigated whether central 5-HT$_7$ receptors are also involved in the vagal bradycardia evoked by baroreceptor and chemoreceptor reflexes.

Male Sprague-Dawley rats (300–350 g) were anaesthetised with α-chloralose (80 mg kg^{-1} I.V. and 15 mg kg^{-1} when required), atenolol pretreated (1 mg kg^{-1} I.V.), neuromuscularly blocked (α-bungarotoxin 150 μg kg^{-1} I.V.; Jones *et al.* 2002), mechanically ventilated, and instrumented to record BP, phrenic (PNA) and renal nerve activity (RNA), and ECG (R-R interval). Depth of anaesthesia was assessed by the stability of BP and HR following a noxious stimulus. Baroreceptor afferents were activated by

stimulating an aortic depressor nerve (ADN; 5 s, 40 Hz, 0.1 ms, 0.1–1 mA). Chemoreceptor afferents were activated by NaCN (75–150 μg kg^{-1} I.V.). After 3 control reflexes, either SB-269970 (100 μg kg^{-1}) or saline was given intracisternally (I.C.; 10 μl). Baroreflexes were repeated at 5, 15, 25 min, and chemoreflexes at 10, 20, 30 min. At the end of experiments animals were humanely killed with an overdose of pentobarbitone.

	Control	5 min	25 min
ADN + saline	53±15	54±14	38±6
ADN + SB-269970	50±4	24±6*	24±8
	Control	**10 min**	**30 min**
NaCN + saline	22±2	29±3	27±2
NaCN + SB-269970	30±4	11±2**	20±7

Table 1. Effect of intracisternal saline and SB-269970 on changes in R-R interval (ms; mean ± S.E.M.) evoked by ADN stimulation and NaCN injection (n = 5 all groups). *$P < 0.05$, **$P < 0.01$, 2-way ANOVA followed by least significant difference test.

SB-269970 significantly attenuated both baro-and chemoreflex increases in R-R interval (Table 1), as well as the chemoreflex increase in RNA (42 ± 6% of control). It had no effect on baseline BP, HR or RNA.The data indicate that central 5-HT$_7$ receptors contribute to the activation of cardiac vagal preganglionic neurones by both IXth and Xth nerve afferents.

Hagan JJ *et al.* (2000). *Br J Pharmacol* **130**, 539–548.
Jones GA *et al.* (2002). *Auton Neurosci* **98**, 12–16.
Kellett DO *et al.* (2003). *J Physiol* **551**, 5*P*.
Skinner MR *et al.* (2002). *Br J Pharmacol* **137**, 861–873.

DOK is a BHF Ph.D student.

All procedures accord with current UK legislation

C26

Cardiovascular autonomic dysfunction in neonates with coarctation of the aorta (CoA)

J.W. Polson, N. McCallion, M.A. Tooley, G. Thorne, S. Kasparov, J.F.R. Paton and A.R. Wolf

*Division of Cardiac, Anaesthetic and Radiological Sciences, & *Department of Physiology, Bristol Heart Institute, University of Bristol, Bristol, UK*

CoA is a common congenital malformation with variable narrowing of the proximal descending thoracic aorta. Surgical correction is the accepted medical treatment, and delayed repair is associated with abnormalities of arterial pressure (AP) homeostasis and early death. It was hoped that neonatal repair would prevent later cardiovascular complications, but recent data have indicated that despite an early and successful anatomic repair, 30% of patients still subsequently develop hypertension (O'Sullivan *et al.* 2002). The aetiology is unclear, but may involve changes in cardiovascular autonomic regulation during the perinatal period. Two important indices of cardiovascular autonomic function are baroreceptor reflex sensitivity (BRS) and heart rate variability (HRV). Neither of these have been examined in the neonate with CoA. In order to understand the development of hypertension in patients with CoA, we have examined BRS and HRV in neonates at first presentation.

After local ethical committee approval and informed parental consent we recruited 6 neonates undergoing elective CoA repair and compared them with 10 healthy newborn infants. AP was measured non-invasively using photoplethysmography by means of adapting a Portapres (Finapres Medical Systems, Netherlands) for use in infants by placing the cuff around the infant's right wrist. Accuracy of the recording was confirmed initially by comparison with measurements made using an arterial line. ECG was recorded using a standard three lead configuration. AP and ECG waveforms were sampled at 1–5 kHz and recorded continuously for 20–30 min while infants were in active sleep. Spontaneous BRS was measured using a modification of the time sequence method of Oosting *et al.* (1997). HRV was examined using standard time domain measurements. Results are expressed as mean ± S.E.M. All infants were under 5 weeks of age.

We found that there was good correlation between Portapres and arterial line measurements of AP (mean 53.2 ± 0.5 v 55.8 ± 0.2 mmHg, respectively), although pulse pressure was lower on Portapres measurements (30.2 ± 1.2 v 38.7 ± 2.7 mmHg). Systolic AP was higher in CoA than controls (79.5 ± 4.6 v 68.8 ± 2.4 mmHg). BRS was reduced by 37% in CoA compared to controls (9.1 ± 1.9 v 14.4 ± 1.6 ms/mmHg, respectively), and HRV was similarly reduced: the range over which 95% of R-R intervals fell was 89.9 ± 11.2 v 148.0 ± 9.1 ms, and the standard deviation of the R-R intervals was 22.5 ± 2.8 v 37.2 ± 2.5.

These data indicate that cardiovascular autonomic regulation is abnormal in infants with preoperative CoA.

O'Sullivan *et al.* (2002). *Heart* **88**, 163–188.
Oosting *et al.* (1997). *J Hypert* **15**, 391–399.

We gratefully acknowledge the support of B. Braun Medical Ltd.

All procedures accord with current local guidelines and the Declaration of Helsinki

C27

Cellular phenotype specificity of viral vectors delivered into cardiovascular control centres in the brainstem

T. Lonergan*, A.G. Teschemacher†, J.F.R. Paton* and S. Kasparov*

** Department of Physiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD.† . Department of Pharmacology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK*

Viral vectors are widely used as tools for exploring gene function. Despite this, very little is known about the cellular phenotype specificity of the promoters used in these vectors within the central nervous system. It is often assumed that all neuronal populations will express a transgene if it is driven by either the human cytomegalovirus (hCMV) promoter, the most commonly used, or the synapsin1 promoter that is active only in neurones. With our interest in the role of central catecholaminergic neurones in cardiovascular control, we have compared the expression of green fluorescent protein in the dorsal vagal complex when driven by three different promoters operating within an adenoviral backbone. The promoters were: hCMV, the synapsin1 promoter and PRSx8 (a synthetic promoter based on Phox2 binding motifs, which determine the catecholaminergic phenotype; see Hwang *et al.* 2001).

Male Wistar rats (75–150g) were anaesthetised (ketamine, 60 mg kg^{-1} and medetomidine, 250 μg/kg, ip) and 2 bilateral injections (1 μl each, 0.5 μl/min) of adenoviral vectors were made into the dorsomedial medulla, including the A2 region (AdhCMVeGFP: 2.7×10^{10} pfu/ml, $n = 3$; AdPRSx8eGFP: 2.2×10^{10} pfu/ml, $n = 3$; AdSYN1eGFP-WHE: 4.4×10^{7} pfu/ml, $n = 3$), or A1 region (AdPRSx8eGFP, $n = 3$) or A6 region (bilateral, AdPRSx8eGFP, $n = 3$). The rats were allowed to recover for 5 days and were then deeply anaesthetised

(pentobarbitone 100 mg kg^{-1}, ip), perfused and brainstem sections processed for dopamine β-hydroxylase (DBH, an enzyme characteristic for catecholaminergic neurones).

Following injection of AdhCMVeGFP or AdSYN1eGFP-WHE no expression of eGFP was observed in DBH-immunoreactive neurones but adjacent dorsal vagal-and hypoglossal motoneurones did exhibit green fluorescence. When the PRSx8 promoter was used in the A2 and A1 regions 26 ± 3 % and 56 ± 11 % of DBH-immunoreactive neurones expressed eGFP, respectively (M ± S.E.M.). The morphology of the remaining eGFP expressing neurons was consistent with that of the cholinergic motoneurons of the dorsal motonucleus of the vagus and nucleus ambiguus, which are also known to express Phox2. In contrast, 96 ± 3 % GFP neurons in the A6 were DBH-immunoreactive, suggesting high selectivity in this region.

Thus, it appears that while the hCMV and synapsin promoters do express in some neurones they are not useful for targeting catecholaminergic neurones. On the other hand, using PRSx8 promoter we achieved transgene expression in all catecholaminergic groups tested and therefore believe this particular vector will be a useful tool for studying the role of these neurones in cardiovascular control. However, further refinements of this vector may be necessary to avoid expression in other phenotypes.

Hwang *et al.* (2001). *Human Gene Therapy* **12**, 1731–1740.

Financial support: WT (AL/069061), Royal Society (23697), BBSRC (7/JE616459), BHF (RG/02/011).AdhCMVeGFP or AdSYN1eGFP-WHE vectors were kindly supplied by Prof. J. Uney (UOB)

All procedures accord with current UK legislation

C28

Advanced method of analysis of the beat-to-beat cardiovascular variability for the investigation of the static and dynamic properties of the baroreflex function

G. Gulli, V. Cooper, V. Claydon and R. Hainsworth

Institute of Cardiovascular Research, University of Leeds, UK

In humans the baroreceptor reflex has been studied noninvasively from the relationship between the cardiac interval and the preceding value of arterial blood pressure. This can be studied as a response to an induced perturbation or as responses of 'spontaneously' occurring pressure changes. The methods provide a measure of the gain which is essentially a static value. Further information more on the dynamic characteristics of the reflex could be obtained from the blood pressure-pulse interval relationship by examining, in addition to the gain, the reflex delay. We also wished to examine those 'non-baroreflex' sequences in which changes in pulse interval preceded rather than followed the changes in pressure. We present a self made program (matlab), which performs the analysis of RR period (ECG) and systolic arterial pressure (SAP, Finapres) changes of time series recorded during 20 min supine and 60° head-up-tilt (HUT). RR-SAP relationship analyses were accepted only when there was a linear correlation ($r^2 > 0.85$) between 4 consecutive heart beats. In addition to the reflex gain, we determined the delay in the response of the baroreflex. For each series the lag in the baroreflex response was determined identifying the beat from which the highest correlation between RR-SAP changes was found. Likewise, analysis of lag was performed to detect the delay of SAP changes due to RR changes. By filtering the time series, analysis was performed on the entire time series, and on the LF (0.04–0.15 Hz) and HF components (0.17–0.4 Hz.) separately. The experiments were carried out after approval from the local ethics committee.

The results obtained in 9 healthy volunteers, when the entire time series was analysed, showed that the optimal baroreflex response occurred with not constant beats of delay. In supine it usually occurred with zero beats of lag. In HUT it occurred with one or more beats of lag. SAP changes due to RR changes (non baroreflex) occurred mostly with two-three beats of delay and this remained constant also in HUT. In HUT there were more 'baroreflex' and less 'non baroreflex' sequences than in supine. When the LF and HF ranges were considered separately, similar results were obtained. Preliminary results on patients with postural-related syncope and poor tolerance in an orthostatic stress test were similar, though in supine there was a prevalence of "baroreflex" sequences with one or more beats of delay. Twelve patients with diabetic autonomic neuropathy were also considered. In these patients it was not possible to detect a clear pattern in the RR-SAP variability.

These preliminary results confirm the presence of a dynamic, rather than only a static characteristic of the baroreflex, which should be considered in the evaluation of the baroreflex function.

All procedures accord with current local guidelines and the Declaration of Helsinki

---

C29

Effect of chronic intermittent hypoxia on cardiac baroreflex in rats

D. White and N.H. West

Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada

The rat intermittent hypoxia (IH) model has facilitated investigation of the relation between obstructive sleep apnea and hypertension. However, the effects of IH on the baroreflex have received relatively little attention. The aim of this study was to examine the hypothesis that IH alters baroreflex function, thus contributing to increased blood pressure variability and the development of hypertension.

Wistar rats (IH-rats, $n = 9$) implanted with telemetry devices (halothane anaesthesia, 2 % in O2) for measurement of arterial pressure (MAP) and heart rate (HR) were exposed to IH consisting of oscillations of inspired oxygen fraction from 21 % to 1 % twice per min, 6 h per day for 7 consecutive days. Controls (Sham-IH rats, $n = 6$) were exposed to cycling compressed air at the same flow rates as for IH-rats. On Day 8, animals were killed by CO_2 exposure and their hearts removed. All procedures were approved by the University of Saskatchewan Committee on Animal Care and Supply and are in accordance with the Guidelines of the Canadian Council on Animal Care.

IH-rats showed significant cardiac hypertrophy (0.45 ± 0.01 *vs.* 0.37 ± 0.02 mg/g body weight, left ventricle + septum; 0.15 ± 0.01 *vs.* 0.12 ± 0.01 mg/g body weight, right ventricle; IH *vs.* Sham). MAP was not different between or within groups over the course of the experiment. Day 1 HR did not differ between groups, however IH-rat HR tended to decrease over time ($P = 0.14$), becoming significantly lower than Sham-IH HR on days 5–8 (Day 1: 337 ± 8 *vs.* 354 ± 10 bpm, Day 8: 323 ± 6 *vs.* 361 ± 9 bpm; IH *vs.* Sham). Though not significant, there was a trend for spontaneous baroreflex sensitivity to increase with IH (4.5 ± 0.5 *vs.* 6.3 ± 1.0 msec/mmHg, Day 1 ($n = 9$) *vs.* Day 7 ($n = 7$), $P = 0.10$) as determined by application of the sequence method to daily recordings of MAP and HR (Bertinieri *et al.* 1988). Values are mean ± S.E.M. and were compared using unpaired Student's *t* tests and repeated measures ANOVA where appropriate. $P < 0.05$ was considered significant.

Contrary to previous reports (Fletcher, Bao, and Li, 1999), IH did not induce hypertension in this study. The lowering of resting HR in IH-rats without a change in MAP may reflect a change in baroreflex set point. Spontaneous baroreflex analysis was limited by the relatively few sequences meeting analysis criteria. Continued studies are required to determine whether the trends in baroreflex function inferred are in fact a significant consequence of IH exposure.

Bertinieri G *et al.* (1988). *Am J Physiol* **254**, H377–H383.
Fletcher EC Bao G & Li R (1999). *Hypertension* **34**, 309–314.

This work was supported by the Natural Sciences and Engineering Research Council of Canada.

All procedures accord with current national and local guidelines

---

C31

Discharge of medullary pacemaker neurones underlies the neurogenesis of the gasp in the rat

Julian F.R. Paton and Walter M. St-John*

*Department of Physiology, School of Medical Science, University of Bristol, Bristol, UK and *Department of Physiology, Dartmouth Medical School, Lebanon, NH, USA*

Eupnea and gasping differ in multiple aspects, including the mechanisms of neurogenesis. A ponto-medullary neuronal circuit is hypothesized to generate eupnea, whereas the gasp is generated by the discharge of medullary "pacemaker" neurones. These pacemaker mechanisms, which are likely to be suppressed during eupnea, are released in severe hypoxia to generate the gasp. We hypothesized that medullary neuronal activities, having discharge patterns consonant with generating the gasp, would continue periodic bursting following a blockade of synaptic transmission.

Activities of the phrenic nerve and single respiratory-modulated neurones, located in the ventrolateral medulla, were recorded extracellularly with glass microelectrodes (3M NaCl; 7–19 MΩ). Recordings were from the decerebrate, *in situ* preparation of the juvenile rat (see Paton 1996, for full details and initial anaesthesia). During eupnea, the preparation was perfused with a solution equilibrated with a hyperoxic-normocapnic gas mixture (95% oxygen and 5% carbon dioxide). Gasping was produced by switching to a separate perfusate equilibrated with a hypoxic (5% oxygen) hypercapnic (8% carbon dioxide) gas mixture.

In eupnea, neurones had "inspiratory", "expiratory" or "phase-spanning" patterns. In gasping, many neuronal activities either discharged during the phrenic burst or ceased firing. However, others that were inspiratory or expiratory-inspiratory phase-spanning in eupnea, became "pre-inspiratory" in gasping, with discharge commencing before the phrenic burst. These "pre-inspiratory" neurones were recorded following a blockade of synaptic transmission. This blockade was produced by adding antagonists to the perfusate to block both inhibitory (glycine and $GABA_A$ receptors: strychnine, 1 μM; bicuculline-free-base, 20 μM respectively) and excitatory (NMDA and non-NMDA receptors: (RS)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid or CPP, 10 μM; kynurenic acid, 2.5–6.0 mM) neurotransmission. Kynurenic acid was added in increments until phrenic activity ceased. These concentrations were based upon other studies in which synaptic events were abolished in intracellularly recorded respiratory-modulated hypoglossal neurones. In 13 neurones tested, 8 exhibited periodic bursting during hypoxia. Of these 8, four neurones also displayed bursting in hyperoxia. The five other neurones remained silent.

We conclude that neuronal activities, compatible with generating gasping, can discharge by intrinsic pacemaker mechanisms. These neuronal activities may underlie the neurogenesis of the gasp.

Paton JFR (1996). *J Neurosci Methods* **65**, 63–68.

British Heart Foundation and NIH funded research

All procedures accord with current UK legislation

C32

Evidence that the Kv3.1b subunit isoform contributes to action potential repolarisation in neurones within the nucleus of the solitary tract in rat

Mark L. Dallas, Susan A. Deuchars, David I. Lewis and Jim Deuchars

School of Biomedical Sciences, University of Leeds, Leeds LS2 9NQ, UK

The voltage gated potassium channels Kv3 subfamily has been specifically implicated in the fast spiking neuronal phenotype, facilitating brief action potentials (Rudy *et al.* 1999). We have localised Kv3.1b immunoreactivity within specific regions of the nucleus of the solitary tract (NTS, Deuchars & Atkinson, 2001) and shown neurones within these regions to be sensitive to 4-AP and TEA (Dallas *et al.* 2002). Since no pharmacological tools are available to distinguish between the Kv3.1 isoforms we sought to determine the specific role of a Kv3.1b subunit, using a novel approach of intracellular application of a Kv3.1b specific antibody.

Male Wistar rats (15–21days) were humanely killed by anaesthetising with sodium pentobarbitone (120 mg kg^{-1}, I.P.) followed by transcardial perfusion with sucrose aCSF and subsequent decapitation. Whole cell patch clamp recordings were made from neurones within the NTS ($n = 7$) and the dorsal vagal nucleus (DVN, $n = 7$) at room temperature. The primary antibody raised against a Kv3.1b channel subunit (1:1000, Alomone Labs) was added to the intracellular solution contained within the patch pipette. In NTS neurones this resulted in progressive prolongation of the action potential duration (from 4.6 ± 0.7 ms to 7.7 ± 0.8 ms at 30 min, mean ± S.E.M.; $P < 0.05$, Student's paired t test). After 30 min 4-AP (30μM) produced a further significant increase in the AP duration (7.7 ± 0.8 ms to 10.1 ± 1.1, $P < 0.05$), which was reversed upon washout of the 4-AP. Repeated applications of 4-AP revealed a progressive blockade due to the antibody since at 65 min 4-AP had no further effect on the action potential duration (11.2 ± 0.8 ms to 11.4 ± 0.7 ms, $P > 0.05$). In the DVN neurones, application of the antibody to the pipette did not lead to a significant increase in the AP duration (5.8 ± 0.6 ms to 5.6 ± 0.3 ms, $P > 0.05$). After 30 and 65 mins application of 4-AP and TEA did not significantly alter the electrophysiological characteristics of the neurones (4-AP; 5.6 ± 0.3 ms to 5.7 ± 0.8 ms, TEA; 5.75 ± 0.6 to 5.5 ± 0.9 ms, $P > 0.05$).

These data suggest that the introduction of a Kv3.1b antibody into the neurone specifically disrupts the channel subunit and that this subunit has a role in the action potential repolarisation in NTS neurones.

Dallas ML *et al.* (2002). *J Physiol* **544.P**, 33*P*.
Deuchars J & Atkinson L (2001). *Soc Neurosci Abstr* **169.6**.
Rudy B *et al.* (1999). *Ann NY Acad Sci* **868**, 1–12.

The University of Leeds and the Wellcome Trust supported this work

All procedures accord with current UK legislation

C33

Differential effect of intracellular Mg^{2+} on voltage-gated K^+ (K_V) currents in rat conduit pulmonary arterial smooth muscle cells (PASMCs)

P. Tammaro and S.V. Smirnov

Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, UK

The role of extracellular Mg^{2+} in the regulation of smooth muscle contractility and reactivity is relatively well characterised. The role of intracellular Mg^{2+} ($[Mg^{2+}]_i$), maintained between 0.5 and 1 mM), in the control of vascular function, however, is less known (e.g. Altura *et al.* 1993). Recent evidence suggests that $[Mg^{2+}]_i$ can rapidly change in response to various vasoactive substances (Touyz & Schiffrin, 1996; Zheng *et al.* 2001). Also, Gelband *et al.* (1993) previously reported that K^+ channels in canine PASMCs were inhibited by $[Mg^{2+}]_i$. In this study, the effect of $[Mg^{2+}]_i$ on voltage-dependent properties of two types of K_V currents, I_{K1} and I_{K2} which we have recently described in rat conduit PASMCs (Smirnov *et al.* 2002), was characterised using the whole cell patch clamp technique.

Male Wistar rats (225–275 g) were humanly killed and PASMCs isolated as described in Smirnov *et al.* (2002). Experiments were performed at room temperature in the presence of 1 μM paxilline and 10 μM glibenclamide to block BK_{Ca} and ATP-senstivie K^+ currents, respectively. Cells were perfused with a pipette solution containing either 0, 5 or 10 mM $MgCl_2$ to vary $[Mg^{2+}]_i$. Comparison of the maximal whole-cell conductance and steady-state activation (calculated from the I-V relationships) and inactivation (measured at +60 mV after 10 s conditioning depolarisations) for I_{K1} and I_{K2}, which is thought to be carried through K_V1 and K_V2 channel subtypes respectively (Smirnov *et al.* 2002), showed a differential sensitivity of these two types of K_V currents to variation in $[Mg^{2+}]_i$.

The maximal conductance for I_{K1} was significantly decreased from 1.14 ± 0.17 nS/pF (mean ± S.E.M., $n = 12$, 0 mM $MgCl_2$) to 0.42 ± 0.06 nS/pF ($n = 11$, 10 mM $MgCl_2$, $P < 0.0007$, unpaired Student's t test), while that for I_{K2}, did not change significantly (0.13 ± 0.01 and 0.11 ± 0.01 nS/pF in 12 and 14 PASMCs studied in 0 and 10 mM $MgCl_2$, respectively). On the other hand, the mid-activation potential (V_a) for I_{K2} was significantly shifted to more negative membrane potentials from 4.1 ± 2.8 mV ($n = 13$, 0 mM $MgCl_2$) to −8 ± 2.1 mV ($n = 14$, 10 mM $MgCl_2$, $P < 0.002$), while V_a for I_{K1} was not affected. However, the opposite effect on the mid-inactivation potential (V_h) was observed, An increase of $MgCl_2$ concentration in the pipette from 0 to 10 mM shifted the I_{K1} inactivation dependency to the left by 26 mV from −23 ± 2 mV ($n = 6$)to −49 ± 5 ($n = 6$) respectively ($P < 0.008$), while V_h for I_{K2} was only marginally affected −54 ± 2 mV *vs.* −59 ± 4 mV in 10 and 6 PASMCs studied in 0 and 10 mM $MgCl_2$, respectively, $P > 0.24$).

Our findings demonstrate that two distinct K_V channel types in PASMCs are differentially regulated by $[Mg^{2+}]_i$. A comparison of hypothetical K_V 'window' current predicts that I_{K1} is more sensitive to changes in $[Mg^{2+}]_i$ than I_{K2} in the voltage range close to the cell resting membrane potential.

Altura BM *et al.* (1993). *Miner Electrolyte Metab* **19**, 323–36.

Gelband CH *et al.* (1993). *Circ Res* **73**, 24–34.

Smirnov SV *et al.* (2002). *J Physiol* **538**, 867–78.

Touyz RM & Schiffrin EL (1996). *J Biol Chem* **271**, 24353–58.

Zheng T *et al.* (2001). *Eur J Pharmacol* **413**, R1–R3.

This work was supported by the British Heart Foundation (grant FS/2000013).

All procedures accord with current UK legislation

C34

Release of nitric oxide (NO) from the hindlimb of the anaesthetized rat in response to systemic hypoxia and adenosine infusion

Clare J. Ray and Janice M. Marshall

Department of Physiology, Medical School, University of Birmingham, Birmingham B15 2TT, UK

The hindlimb vasodilatation evoked in the rat by systemic hypoxia is mediated by adenosine acting at the A_1 receptors and is attenuated by the NO synthase (NOS) inhibitor nitro-L-arginine methyl ester (L-NAME; Bryan & Marshall, 1999*a,b*). Further studies showed the adenosine-mediated component of hypoxia-induced hindlimb dilatation and of proximal arterioles can be restored after NOS blockade providing a background level of NO is restored with an NO donor whereas the hypoxia-induced dilatation of terminal arterioles was not restored (Edmunds *et al.* 2003; Edmunds & Marshall, 2003). These studies raised the question of whether hypoxia-and adenosine-induced vasodilatation is simply NO-dependent or mediated by an increase in NO synthesis. We have now investigated the release of NO from the rat hindlimb by measuring NO concentration ([NO]) in the blood by reduction of plasma nitrate (NO_3^-) to nitrite (NO_2^-) followed by electrochemical measurement of [NO] following the reduction of NO_2^- by a 0.1M H_2SO_4/KI solution.

Rats were anaesthetized with Saffan (7–12 $mgkg^{-1}hr^{-1}$ I.V.) and humanely killed by anaesthetic overdose. Arterial and venous blood samples (100 μl) were taken from the femoral artery and vein before and in the 5th min of systemic hypoxia (8% inspired O_2) or infusion of adenosine (1.2 $mgkg^{-1}min^{-1}$, I.A.). In Group 1 ($n = 8$) and Group 2 ($n = 6$), systemic hypoxia and adenosine infusion evoked vasodilatation that was significantly attenuated by L-NAME (10 $mgkg^{-1}$ I.V.) and by the adenosine A_1-receptor antagonist DPCPX (0.1 $mgkg^{-1}$ I.V.; see Bryan & Marshall, 1999*a,b*). In Group 1, systemic hypoxia and adenosine infusion evoked a significant increase in venous-arterial [NO] difference ($[NO]_{v\text{-}a}$) from −1.4 ± 0.9 to 6.6 ± 1.6*** and from 2.3 ± 0.8 to 8.4 ± 1.8** nM respectively (mean ± S.E.M.; ***, **, *$P < 0.0001$, 0.001, 0.05 ANOVA for repeated measures with Fisher's post hoc test), which was abolished in the presence of L-NAME, from −0.72 ± 0.9 to −0.87 ± 0.7 and 0.72 ± 0.8 to −0.97 ± 1.1 nM respectively. In Group 2, DPCPX abolished the increase in $[NO]_{v\text{-}a}$ evoked by systemic hypoxia (from −4.2 ± 1.8 to 12.5 ± 3.7** to −0.63 ± 2.6 to 3.3 ± 2.9 nM) and reduced by ~50% the increase in $[NO]_{v\text{-}a}$ evoked by adenosine infusion (from 1.1 ± 1.5 to 23.7 ± 13.7* to −0.43 ± 2.9 to 11.6 ± 5.9 nM).

Thus, we have provided the first direct evidence that the muscle vasodilatation of systemic hypoxia that is mediated by adenosine acting at A_1 receptors, is accompanied by an increase in the release of NO.

Bryan PT & Marshall JM (1999*a*). *J Physiol* **514**, 151–162.

Bryan PT & Marshall JM (1999*b*). *J Physiol* **514**, 163–175.

Edmunds NJ *et al.* (2003). *J Physiol* **546**, 521–527.

Edmunds NJ & Marshall JM (2003). *J Vasc Res* **40**, 68–76.

All procedures accord with current UK legislation

C35

Genomic responses to chronic systemic hypoxia in skeletal muscle of rats *in vivo*

Katie E. Glen, Janice M. Marshall, D. Candinas* and Deborah M. Stroka*

*Department of Physiology, Medical School, University of Birmingham, Birmingham B15 2TT, UK and *VCHL, University Hospital Bern, Switzerland*

Our recent studies on rats showed that in the first 1–7 days of chronic hypoxia there is vasodilatation (Walsh & Marshall, 1999) and by 3 weeks there is capillary angiogenesis (Deveci *et al.* 2001) in skeletal muscle. In the present study we have investigated the genomic events that may underlie these changes.

Experiments were performed on chronically hypoxic (CH) rats housed in a hypoxic chamber at 12 % O_2 for 2, 6, 12 h and 1, 3, 7 and 14 days ($n = 6$ in each case) and on 6 normoxic (N) rats that breathed air. Under anaesthesia (Sagatal 60 mg kg^{-1}, Tibialis Anterior (TA; mainly glycolytic), Soleus (SOL; oxidative) and Spinotrapezius (SP; mixed fibre types) muscles were removed and frozen in liquid N_2-cooled 2-methylbutane. All animals were humanly killed by anaesthetic overdose. mRNA was quantified by real-time PCR using probes and primers to VEGF, iNOS and eNOS. Values are expressed as fold increases (mean ± S.D.) in CH relative to N rats. All changes described below were significant ($P < 0.05$) when analysed by ANOVA followed by Dunnett's post hoc test when appropriate.

In SOL there was an increase in iNOS, VEGF and eNOS mRNA expression. iNOS mRNA showed a time-dependent increase from 2hr reaching a 6.2 ± 1.7-fold increase at 1day and remained 3-fold greater than N rats at 3, 7 and 14 days. VEGF mRNA increased from 1–14days reaching a maximum 4.8 ± 1.3-fold increase at 3days, but falling to 2.3 ± 0.9-fold at 14days. eNOS mRNA was increased by 2.2 ± 1.0-fold at days 7 and 14.In TA, there was a 2–2.5-fold increase in iNOS mRNA at all time points. Similarly VEGF mRNA showed a prolonged increase reaching 2.0 ± 0.8-fold at 6hr. eNOS mRNA was similar to that seen in SOL: 2.0 ± 0.6-fold increase at day 7.In SP, iNOS mRNA showed a similar time-dependent increase to SOL: 3.7 ± 1.3-fold, at 1day. Any change in VEGF mRNA did not reach statistical significance. In contrast to SOL and TA, eNOS mRNA in SP was increased earlier: at 6hr by 2.3 ± 1.0-fold.These results suggest chronic hypoxia induces genomic responses in skeletal muscle of different time-dependencies and magnitudes in muscles of different fibre type. Increases in iNOS and VEGF mRNA were particularly pronounced and prolonged in SOL; this may be consistent with capillary angiogenesis being more substantial in oxidative SOL than in TA (Deveci *et al.* 2001). The increases in iNOS mRNA in all muscles and the sustained increase in eNOS mRNA in SP is consistent with tonic NO-dependent dilatation (Walsh & Marshall, 1999).

Deveci D *et al.* (2001). *Am J Physiol* **281**, H241–H252.

Walsh MP & Marshall JM (1999). *J Physiol* **515.P**, 144P.

We gratefully acknowledge the support of the British Heart Foundation, UK.

All procedures accord with current UK legislation

C36

Altered cotransmitter contributions to sympathetic vasoconstriction of tail artery in streptozotocin diabetic rats

A. Donnelly, S. Roe, C.N. Scholfield and C.D. Johnson

Department of Physiology, Queen's University, Belfast BT9 7BL, UK

Diabetes mellitus is associated with numerous cardiovascular complications including autonomic neuropathy, leading to orthostatic hypotension. Changes taking place in sensitivity to exogenous noradrenaline are well documented (Weber *et al.* 1996) but contributions of other sympathetically released cotransmitters are unclear. In this study we show that vascular responses mediated by ATP in the rat tail artery show distinct changes in the diabetic state.

Sprague-Dawley rats (8 week old, male) were made diabetic by intraperitoneal injection (60 mg kg^{-1}) of streptozotocin and maintained for a further 12 weeks in accordance with UK legislation. Animals were killed by cervical dislocation. Injected animals having a blood glucose of less than 10 mM/l were used a controls ($n = 9$, 492 ± 26 g, mean ± S.E.M.; blood glucose 7 ± 1 mM) while those with higher values were deemed to be diabetic ($n = 12$, 275 ± 12 g, blood glucose 39 ± 2 mM). Tail arteries were excised, endothelium removed and rings cut into 3–5 mm lengths. Isometric contractions were measured. Noradrenaline (0.1 nM–100 μM) and ATP (1 nM–10 μM) were bath applied and dose–response curves constructed and responses examined to either 60 mM KCl or electrical field stimulation by trains of 1–100 impulses (at 20 Hz, 1 ms pulses, supra-maximal voltage). Electrically-evoked responses were examined in the absence or presence of non-specific α-adrenoceptor antagonist, phentolamine (1 μM), or P2 purinoceptor antagonist, suramin (100 μM). Electrically-evoked responses were abolished in the presence of tetrodotoxin (1 μM) or guanethidine (10 μM), indicating their sympathetic origin.

Bath applied noradrenaline and ATP were both more potent in diabetic than non-diabetic arteries (diabetic *vs.* non-diabetic, ED_{50} for noradrenaline: 0.28 ± 0.07 *vs.* 2.93 ± 1.0 μM: $P < 0.005$, unpaired t test). In contrast, there were no differences in KCl constrictions between diabetic and non-diabetic rats (0.94 ± 0.2 g and 0.85 ± 0.07 g respectively; unpaired t test) suggesting the enhanced vasoconstrictor responses were not due to changes in smooth muscle contractility. Nerve stimulation produced contractions which were similar in both diabetic and non-diabetic arteries. In non-diabetic arteries, these were reduced by suramin which was dependent on train impulse number, ie, 58 ± 12 % depression with 2 pulses and 27 ± 13 % 100 impulses). However, in diabetic arteries, depression with suramin was greater for longer pulse trains (depressions of 54 ± 12 % and 68 ± 10 % for 2 and 100 pulse trains; one way ANOVA and SNK, diabetic *vs.* non-diabetic). There were also depressions with phentolamine which was similar for both groups for impulse trains >4 (one way ANOVA).

Thus, we have shown that in diabetic rats, responses to sympathetic activation are preserved but there is a shift towards a greater contribution made by ATP which is dependent on pulse train parameters.

Weber LP *et al.* (1996). *Br J Pharmacol* **118**, 115–122.

AD was funded by a Physiological Society Summer Vacationship

All procedures accord with current UK legislation

PC7

Simulated sleep apnoea in rats. Cardiopulmonary effects of intermittent hypoxia with or without hypertension due to nitric oxide synthase inhibition

G.R. Barer, A.E. Oakley, E.A. Laude*, C.J. Emery*, D. Thwaites-Bee* and D.H. Barer

*Institute for Ageing and Health, Newcastle General Hospital, Newcastle-upon-Tyne NE4 6BE and * Respiratory Medicine, Medical School, Beech Hill Rd., Sheffield S10 2RX, UK*

In children sleep apnoea (SA) causes cardiac and pulmonary vascular changes. SA is also common in the elderly where it is frequently associated with hypertension. In elderly rats (BW~500g), we induced hypertension by inhibition of nitric oxide synthase (NOS) with L-nitro arginine methyl ester (LN, 300 mg ml^{-1} in drinking water) and simulated SA by exposure to intermittent hypoxia (IH: 10 % O2 4h/day, 56 exposures). These were compared with continuous hypoxia (CH: 10 % O2 for 56 days) and normoxia (N) groups, each with and without LN. Systemic (Psyst) and pulmonary (Ppa) arterial pressure were measured under thiopentone anaesthesia (100 mg kg^{-1}, ip).

Table: Comparisons between treatment groups

Group (n)	Psyst (SD) MmHg	Ppa (SD) mmHg	Haemato-crit %	% of EL counts >2μm
N (7)	145 (11)	16 (1.2)	47 (3.6)	16
N + LN (6)	175 (13)	15.7 (2.8)	49 (2.8)	15
IH (6)	148 (12)	21.7 (2.1)	61 (48)	19
IH + LN (6)	193 (11)	16.7 (2.1)	59 (31)	29
CH (6)	139 (8)	31.3 (2.1)	62 (3.4)	33
CH + LN (4)	123 (14)	-	78.5 (3.4)	-

After rats were killed humanely (lethal anaesthetic, ip), heart weights and lung vessels were assessed. The ratio of right to left heart weights (RV/LV+septum) was significantly raised in CH and CH+LN (Student's unpaired *t* test). There were no changes in the number of thick-walled peripheral vessels within the acinus, but we found changes in wall-thickness. Hypoxia causes development of a new elastic lamina (EL) internal to the original, which narrows the lumen, but is unevenly distributed around it. We measured the greatest distance between outer and new inner elastic lamina with an eyepiece graticule and found a clear trend towards thicker walls in hypoxia (Table). The low pulmonary artery pressure (Ppa) in IH+LN was unexpected. We previously found evidence that inhibition of NOS increased the activity of cyclooxygenase (COX) and vice versa (Liu *et al.* 1999). Thus, the low Ppa after NOS inhibition in IH+LN might be due to activity of the COX pathway leading to release of dilators.

Liu X *et al.* (1999). *Exp Physiol* **84**, 907–916.

All procedures accord with current UK legislation

PC8

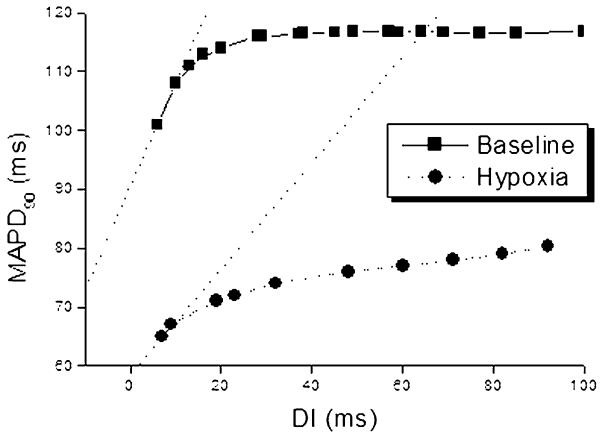
The effect of hypoxia on electrical restitution and inducibility of ventricular fibrillation in the isolated rabbit heart

Vanlata H. Patel, Kieran E. Brack, Suman Kundu, Peter Taggart†, John H. Coote and G. Andre Ng

*Department of Cardiovascular Sciences, University of Leicester, * Department of Physiology, University of Birmingham and †Department of Cardiology, University College Hospital London, UK*

The mechanisms underlying the induction, maintenance and termination of ventricular fibrillation (VF) remain poorly understood, despite being a major cause of sudden death. Signs of myocardial injury are absent in many cases of sudden death even in the presence of coronary artery disease suggesting that mechanisms such as ischaemia or hypoxia rather than infarction may be involved. VF initiation is suggested to be related with the slope of the restitution curve (Garfinkel *et al.* 2000). This study examines the effects of hypoxia on electrical restitution and inducibility of VF in the isolated rabbit heart.

Adult male NZW rabbits (2.0–2.5 kg, $n = 7$) were humanely killed with an overdose of Sagatal (I.V.). Hearts were isolated and Langendorff perfused (35ml min^{-1}, 37 °C). Monophasic Action Potential was recorded from the left ventricular free wall and duration (MAPD) was measured at 90 % repolarisation. Standard restitution was obtained with right ventricular pacing using a 20 beat drive train (S1, 300 ms), followed by an extra stimuli (S2) down to the effective refractory period (ERP). S2-MAPD were plotted against preceding diastolic intervals (DI) and fitted to an exponential curve with maximum slope measured. VF threshold (VFT) was determined as the minimum current required to induce VF using a train of 30 stimuli (30 ms interval). Measurements were made during Tyrode perfusion bubbled with 95 %O_2/5 %CO_2 (Baseline) followed by Tyrode perfusion bubbled with 95 %N_2/5 %$C0_2$ (Hypoxia). Results (mean ± S.E.M.) were analysed using ANOVA.



Hypoxia significantly ($P < 0.001$) reduced heart rate (165±8 to 116±4 bpm), left ventricular pressure (94 ± 6 to 45±7 mmHg), aortic perfusion pressure (58±3 to 42±4 mmHg), ERP and maximum MAPD ($MAPD_{Max}$). VFT was significantly lower whilst the restitution curve was flatter (Fig. 1) and maximum slope significantly decreased during hypoxia (table).

	Baseline	Hypoxia	*p* value
ERP (ms)	122.9±5.1	85.7±3.4	<0.001
VFT (mA)	5.6±0.8	0.6±0.1	<0.001
$MAPD_{90}$ (ms)	134.2±9.4	98.9±8.6	<0.05
Slope	1.4±0.3	0.5±0.1	<0.05

Table: Effect of hypoxia on ventricular electrophysiology

Electrical restitution curve is flatter during hypoxia with less reduction in MAPD at short diastolic intervals when compared to baseline. This suggests that the increased susceptibility of the heart to VF during hypoxia cannot be explained by the slope of the restitution curve alone.

Garfinkel A *et al.* (2000). *Proc Natl Acad Sci U S A* **97**, 6061–6066.

This study is supported by the British Heart Foundation

All procedures accord with current UK legislation

PC9

Systemic P2 receptor blockade attenuates increase in body temperature evoked by lipopolisaccharide in conscious rats

V.N. Gourine, D.M. Poputnikov, E.V. Melenchuk, A.V. Gourine and K.M. Spyer

*Institute of Physiology, National Academy of Sciences of Belarus, Minsk 220725, Belarus and *Department of Physiology, Royal Free and University College London Medical School, London NW3 2PF, UK*

Extracellular ATP acting through ionotropic P2X and metabotropic P2Y receptors, acts as a signalling molecule in the brain and periphery and has numerous physiological functions (Burnstock, 1999; North, 2002). When its role in regulation of body temperature (T_b) during infection is considered the ability to induce a release of cytokines seems to be potentially very significant. We have shown previously that ATP acting on P2 receptors in the CNS plays an important role in thermoregulation during fever (Gourine *et al.* 2002). In this study we investigated the effects of systemic P2 receptor blockade on regulation of T_b during fever.

Experiments were performed in adult male Wistar rats (280–350 g) and were approved by the Institutional Animal Care and Use Committee (in Minsk). Rats were anaesthetised (ketamine [87.0 mg kg^{-1}] + xylazine [13.0 mg kg^{-1}]) and a telemetry transmitter was implanted into the abdomen for monitoring of T_b. After a 7-day recovery period, fever was induced by intraperitoneal injection of *E.coli* lipopolisaccharide (LPS; 50 μg kg^{-1}). Effects of intraperitoneal (I.P.) injection of the P2 receptor antagonists suramin (5, 25 and 100 mg kg^{-1}), PPADS (5 and 25 mg kg^{-1}) or saline on T_b during fever were determined. The rat was humanely killed by overdose of anaesthetic at the end of the experiment.

Following I.P. injection of LPS a fever reached a maximal T_b (around 38.5°C) 3 h after the injection. Dose-dependent attenuation of fever was observed when suramin and PPADS were injected I.P. before administration of LPS. Three h after the administration of LPS T_b of rats pretreated with suramin (100 mg kg^{-1}) was 37.0 ± 0.4°C (mean ± S.E.M.; $n = 11$) some 1.6°C lower that the T_b of febrile rats injected with saline (38.6 ± 0.2°C, mean ± S.E.M.; $n = 7$; $P = 0.018$, ANOVA, post hoc Fisher's test). Similarly, 3 h after the administration of LPS T_b of rats injected with PPADS (25 mg kg^{-1}) was 37.9 ± 0.3°C (mean ± S.E.M.; $n = 7$) some 0.7°C lower that the T_b of LPS-treated rats injected with saline (38.6 ± 0.1°C, mean ± S.E.M.; $n = 15$; $P = 0.017$, ANOVA, post hoc Fisher's test). Both P2 receptor antagonists had no effect on T_b in afebrile animals.

These results indicate that in the periphery ATP-mediated purinergic signalling may play very significant role in the mechanisms leading to the development of the febrile response. We propose that the attenuation of fever induced by systemic treatment with P2 receptor antagonists results from an inhibition and/or an alteration in LPS-induced cytokine release.

Burnstock G (1999). *Prog Brain Res* **120**, 3–10.

Gourine AV *et al.* (2002). *Br J Pharmacol* **135**, 2047–2055.

North A (2002). *Physiol Rev* **82**, 1013–1067.

This work was supported by The Wellcome Trust.

All procedures accord with current national and local guidelines

PC10

Phenotypic differences in heart rate and heart rate variability using conscious inbred strains of mice

R. Howden and S.R. Kleeberger

National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA

Recent studies have suggested a genetic component to heart rate variability (HRV; Singh *et al.* 2001). Inbred mice are useful to investigate the genetic determinants of complex physiological phenotypes. However, investigation of the genetic determinants of heart rate (HR) and HRV presents methodological difficulties when using a mouse model. It is now possible to avoid the confounding affects anaesthesia on mouse cardiac function (Roth *et al.* 2001) by recording the electrocardiogram (ECG) using radio telemetry (Data Sciences Int., MN, USA) in conscious mice. While respiration cannot be controlled in conscious mice, it can be monitored using whole body plethysmography (Buxco Electronics Inc., NY, USA). ECG recordings can be timed to coincide with periods of consistent breathing rate and depth. The purpose of this study was to investigate possible differences in resting HR and HRV in C3H/HeJ and NZB/BinJ inbred mice, under conditions of consistent breathing rate and depth.

All procedures were approved by the NIEHS Animal Care and Use Committee and conformed closely to the principles of UK animal legislation. Ten mice per strain (8–10 wks; 20–30g) were anaethetised with inhaled isoflorane, and buprenorphin was given (0.1 mg/kg) for analgesia. A 3.8 g radio telemetry transmitter (Data Sciences Int., MN, USA) was implanted in a dorsal subcutaneous tissue pocket. ECG and respiratory function were recorded from individual mice following at least 30 min of acclimation to the whole body plethysmographs or until breathing rate and depth became consistent. Following data collection mice were euthanised humanely. R waves in the ECG waveform were marked and extracted, and arrhythmias were removed. HRV was assessed in the frequency domain using the Lomb periodogram technique (Laguna *et al.* 1998). A significant difference was found in resting HR between C3H/HeJ and NZB/BinJ mice ($P < 0.001$, *t* test) (Table 1). However, not all HRV parameters were significantly different between strains. Differences were found in total power (TP) and in the high frequency (HF) range (0.2–5.0 Hz and 1.5–5.0 Hz respectively; $P < 0.001$, ANOVA). HRV in low frequency range was not different between strains (0.2–1.5 Hz; $P > 0.05$, ANOVA).

Table 1. HR and HRV parameter values from two inbred strains of mice (average±SD, * = significant difference between strains)

	C3H/HeJ	NZB/BinJ
HR (bpm)	610.6±43.7	502.5±45.3*
TP (ms^2·Hz)	3.4±0.4	1.5±0.6*
LF (ms^2·Hz)	1.6±0.2	1.1±0.6
HF (ms^2·Hz)	1.8±0.4	0.4±0.2*

Using the above techniques we identified phenotypic differences in HR and HRV. These results provide evidence for a genetic component in the regulation of resting HR and HRV. Positional cloning is required to assess the extent to which genes contribute to HR and HRV.

Laguna P *et al.* (1998). *IEEE Trans Biomed Eng* **45**, 698–715.

Roth DM *et al.* (2002). *Am J Physiol Heart Circ Physiol* **282**, H2134–H2140.

Singh JP *et al.* (2001). *Auton Neurosci* **90**, 122–126.

All procedures accord with current national and local guidelines

PC11

Development of new software for the automation of analysis of cardiovascular autonomic function from chronic measurements of arterial pressure in conscious rats

Hidefumi Waki*, Kiyoaki Katahira†, Sergey Kasparov*, David Murphy‡ and Julian F.R. Paton*

** Department of Physiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, †Experimental Animal Center, Fukushima Medical University School of Medicine, Fukushima 960-1295, Japan, ‡Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Dorothy Hodgkin Building, Bristol BS1 3NY, UK*

Radio-telemetry is an effective technique for recording pulsatile arterial pressure chronically in conscious, unrestrained rodents (Brockway *et al.* 1991; Waki *et al.* 2003). Mathematical analysis of this signal allows an evaluation of changes in vasomotor sympathetic tone, cardiac sympathetic and cardiac vagal tone (Pagani *et al.* 1986; Cerutti *et al.* 1991). Further, spontaneous alterations in blood pressure and corresponding changes in heart rate (HR) allow the spontaneous baroreflex cardiac gain (sBRG) to be measured (Oosting *et al.* 1997; Waki *et al.* 2003). To date this analysis has been highly time consuming requiring multiple software applications off-line. In this study, we introduce new software to automatically evaluate cardiovascular autonomic function on-line from an arterial pressure signal.

The software consists of 2 basic data elements: 1) acquisition and 2) analysis programs. In the data acquisition program, 4 channels of data can be recorded simultaneously at a sampling rate of up to 4 kHz per channel. The exact timing of data sampling, its duration and frequency of collection can be set for each channel. The data analysis program contains a fast Fourier transform function for power spectral analysis of HR variability as well as a program for measuring sBRG based on the time-series technique described previously (Waki *et al.* 2003). In telemetered rats (see Waki *et al.* 2003 for methods), we obtained chronic measurements of arterial pressure. To validate the new software, we measured changes in the high frequency (HF) power of the pulse interval (PI), which is an index of cardiac parasympathetic activity, before and after vagal blockade (atropine, 1 mg kg^{-1}, I.V.). Low frequency (LF) /HF of PI, which is considered to reflect levels of cardiac sympathetic activity, was also calculated before and after cardiac sympathetic blockade (atenolol, 1 mg kg^{-1}, I.V.). sBRG was measured before and after injection of these drugs.After vagal blockade, HR was significantly increased (324 ± 22 *vs.* 392 ± 10 bpm, mean ± S.D., $P < 0.001$, ANOVA, $n = 4$) while HF power of the PI was significantly decreased (28.5 ± 4.7 *vs.* 16.8 ± 2.3 $msec^2$, $P < 0.001$, $n = 4$). Following subsequent sympathetic blockade, HR and LF/HF of PI was significantly decreased (HR, 392 ± 10 *vs.* 321 ± 12 bpm, $P < 0.001$; LF/HF of the PI, 0.37 ± 0.20 *vs.* 0.15 ± 0.02, $P < 0.05$, $n = 4$). The sBRG, was also decreased by 42 % after vagal blockade (0.69 ± 0.14 *vs.* 0.29 ± 0.05 msec $mmHg^{-1}$, $P < 0.001$, $n = 4$) and decreased further after sympathetic blockade (0.29 ± 0.05 *vs.* 0.17 ± 0.04 msec $mmHg^{-1}$, $P = 0.08$, $n = 4$). These findings in the sBRG were mirrored by measurements using conventional vasoactive drugs (before blockade, 0.63 msec $mmHg^{-1}$; after vagal blockade, 0.27 msec $mmHg^{-1}$; after vagal and sympathetic blockade, 0.12 msec $mmHg^{-1}$).

These results suggest that our software adequately evaluates cardiovascular autonomic function from chronic measurements of arterial pressure that include HR variability and sBRG. We believe that it will be a powerful tool for analysis and quantification of long-term changes in cardiovascular autonomic function in conscious rodents. Additional functions to measure LF component of arterial pressure, which is considered to reflect changes in vasomotor sympathetic tone and respiratory rate, are currently under development.

Brockway BP *et al.* (1991). *Clin Exp Hypertens* **13**, 885–895.

Cerutti C *et al.* (1991). *Am J Physiol* **261**, H1292–1299.

Oosting J *et al.* (1997). *J Hypertension* **15**, 401–410.

Pagani M *et al.* (1986). *Cir Res* **59**, 178–193.

Waki H *et al.* (2003). *J Physiol* **546**, 233–242.

JFRP was supported by the British Heart Foundation.

All procedures accord with current UK legislation

PC12

The influence of breathing 40 % oxygen on the response to maximal voluntary contraction of the forearm in human subjects

G. Fordy and Janice M. Marshall

Department of Physiology, Division of Medical Sciences, The Medical School, Birmingham B15 2TT, UK

It is widely accepted that breathing supplementary O_2 during whole body dynamic exercise, enhances performance (Linossier *et al.* 2000). The aim of the present study was to test the effect of supplementary O_2 on muscle haemodynamics and time to exhaustion in a protocol involving repetitive maximal handgrip exercise.

10 healthy male subjects with age, mass and height of 23.7 ± 0.3 years, 77.8 ± 1.91 kg and 1.79 ± 0.19 m respectively (mean ± S.E.M.) were used with local ethics committee approval. Two different protocols were conducted in a single-blind crossover design. Each protocol consisted of 2 periods of maximal handgrip exercise to exhaustion separated by a 5-minute recovery period with subjects breathing 40 % O_2 during exercise or recovery. Forearm blood flow (FBF) was measured by venous occlusion plethysmography. Arterial blood pressure (ABP) was measured continuously by Finapres.

In protocol 1, post-contraction FBF was lower when subjects breathed 40 % O_2 *during contraction* than when they breathed air throughout: at 15 s after contraction 1, FBF was 24.11 ± 2.13 *vs* 18.64 ± 1.95 and at 15 s after contraction 2 FBF was 25.89 ± 2.13 *vs* 18.40 ± 1.52 ml 100 ml^{-1} min^{-1}*; air *vs* 40 % O_2: * $P < 0.05$ Factorial ANOVA. Time to exhaustion was longer for contraction 1 than contraction 2 in both air-and 40 % O_2-

breathing trials: 254 ± 24.52 *vs* 170.5 ± 20.82 † and 268 ± 21.04 *vs* 170.66 ± 25.29 s † respectively: † $P < 0.05$ Student's paired *t* test.

In protocol 2, post-contraction FBF was not different when subjects breathed 40 % O_2 *during recovery* than when they breathed air throughout for contraction 1 or 2. However, time to exhaustion was lengthened for contraction 2 when subjects breathed 40 % O_2 during recovery: 172.6 ± 32.6 *vs* 119 ± 19.44 s †, contraction 1 *vs* 2, air breathing and 169 ± 28.87 *vs* 158.2 ± 32.27 s, contraction 1 *vs* 2, 40 % O_2 breathing.

These results indicate that breathing 40 % O_2 can have two effects on the response to maximal forearm contraction. When supplementary O_2 was given, even during maximum contraction, forearm post-contraction hyperaemia was reduced indicating additional O_2 delivered during contraction reduced the accumulation of vasodilator metabolites. When supplementary O_2 was given *during recovery*, time to exhaustion of subsequent contraction was lengthened: this may reflect a greater rephosphorylation of ATP from creatine phosphate.

Linossier M *et al.* (2000). *Acta Phys Scand* **168**, 401–411.

All procedures accord with current local guidelines and the Declaration of Helsinki

PC13

The HCN1 ion channel subunit is prominently expressed in somatodendritic domains of neurones in sensory and motor systems

R.E. Brooke, I.J. Edwards, C.J. Milligan, S.A. Deuchars and J. Deuchars

School of Biomedical Sciences, University of Leeds, Leeds LS2 9NQ, UK

Hyperpolarization-activated cyclic nucleotide-gated (HCN) nonselective cation channels in neurones carry cationic currents proposed to generate I_h (Robinson & Siegelbaum, 2003). Several CNS regions express the mRNA for one or more of the 4 HCN subunits. Here we show that immunoreactivity for HCN1 is present in neurones which underlie sensory or motor functions.

Rats (100–200 g, $n = 10$) were injected intraperitoneally with 0.1ml 1 % Fluorogold (Fluorochrome Inc.) and 7 days later were humanely killed by intraperitoneal injection of Sagatal (60 mg kg^{-1}) and transcardial perfusion with 4 % paraformaldehyde/0.1–0.5 % glutaraldehyde. Slices (50 μm) of brainstem, spinal cord, dorsal root ganglia (DRG) and nodose ganglia (NG) were cut on a vibrating microtome and incubated in rabbit anti-HCN1 antibody (Alomone;1:1K) followed by Cy3 conjugated donkey anti-rabbit (Jackson Immunochemicals, 1:500). For double labelling sections were subsequently immersed in a marker for unmyelinated c-fibres, fluorescein conjugated Bandeiraea (Griffonia) Simplicifolia Lectin I (IB4, VectorLabs, 1:100) and/or an indicator of fast conducting myelinated neurones, mouse anti-neurofilament 200 (NF200, 1:1K, Sigma) visualised using a biotinylated conjugated secondary antibody and Streptavidin Alexa^{488}.

HCN1-immunoreactivity (HCN1-IR) was strongly evident throughout the brainstem and spinal cord, including the following motor nuclei: facial, abducens, ambiguus, hypoglossal and ventral horn. These HCN1-IR neurones were also Fluorogold and/or NF200 containing, confirming that they were motor neurones. HCN1-IR was also in neurones of sensory pathways, namely in the cochlear, spinal trigeminal, dorsal column, inferior olivary and lateral spinal nuclei, as well as the area postrema and the dorsal horn. On a cellular level HCN1-IR was present in somatic and dendritic membranes. Primary sensory neurones that were HCN1-IR and Fluorogold containing were detected in the mesencephalic trigeminal nucleus, the NG and DRG. These primary sensory neurones were IB4 negative, but a proportion did contain NF200.

These data show that the HCN subunit HCN1 is present in somato-dendritic regions of neurones which subserve sensory and motor functions. This localisation parallels the expression of I_h in neurones within these populations (e.g. McLarnon, 1995), suggesting that the HCN1 subunit is a contributor to channels mediating these currents.

McLarnon JG (1995). *Prog Neurobiol* **47(6)**, 513–531.

Robinson RB & Siegelbaum SA (2003). *Ann Rev Physiol* **65**, 453–480.

This work was funded by the British Heart Foundation (REB, SAD), Wellcome Trust (CJM) and a Physiological Society vacation studentship (IJE).

All procedures accord with current UK legislation

PC14

PVN-spinal oxytocin neurones selectively increase heart rate in the anaesthetised rat

Z. Yang*, M. Wheatley† and J.H. Coote‡

**The Medical School, University of Nankai, Tianjin, PR China, 300071, †School of Biosciences, The University of Birmingham, Birmingham B15 2TT and ‡Department of Physiology, The University of Birmingham, Birmingham B15 2TT, UK*

In a previous study we showed that activation of spinally projecting neurones in the hypothalamic paraventricular nucleus (PVN) could increase sympathetic activity to the kidney via the release of vasopressin (Yang *et al.* 2002). There was no effect mediated by PVN-spinal oxytocin neurones despite these forming an almost equally abundant projection to the thoracic spinal cord (Cechetto *et al.* 1988). However an early study by Yashpal *et al.* (1987) had revealed that intrathecal (i.t.) application of oxytocin to the spinal cord preferentially increased heart rate. The present study was designed to determine if activation of PVN-spinal neurones could increase heart rate and whether oxytocin neurones were involved.

Eight Wistar rats were anaesthetised with urethane, chloralose mixture (650 mg kg^{-1}, 50 mg kg^{-1}) I.V. and a glass micropipette was inserted into PVN for microinjection of D,L-homocysteic acid (DLH) to activate neurones. Blood pressure and heart rate was recorded and the efficacy of spinal pathways tested by i.t. application of agonists and antagonists via a catheter inserted via the foramen magnum so that its tip lay at T_3 segment. Statistical analysis was performed using Student's two tailed, paired *t* test. Rats were killed by overdose of urethane at end of experiment.

Oxytocin given i.t. (10 μl, 0.02 mM) increased heart rate by 26 ± 5 beats per min (b.p.m.) and this was significantly reduced to 5 ± 1 b.p.m. ($P < 0.01$) when preceded by a highly selective non-peptide oxytocin antagonist L368899 (Yang *et al.* 2002). Similar small increases of 34 ± 5 b.p.m. in heart rate were elicited from a number of sites in the PVN which were significantly reduced to 24 ± 5 b.p.m. ($P < 0.05$). At some of these sites heart rate increases were tested by i.t. application of the glutamate antagonist kynurenic acid (10 μl, 4.0 mM) and were effectively blocked (29 ± 4 b.p.m. before, 2 ± 3 b.p.m. after, $P < 0.01$). In contrast no PVN elicited heart rate increases were significantly changed by prior i.t. application of a vasopressin V_{1a} antagonist (Yang *et al.* 2002) (10 μl, 0.05 mM). The PVN elicited heart rate increases were unaffected by bilateral vagotomy (*22 ± 8* before, 22 ± 4 after, $n = 4$) but were completely blocked by I.V. β 1 adrenoreceptor esmolol (1 mg kg^{-1}) (29 ± 4 before to 6 ± 2 after,

$n = 4$), indicating they were sympathetically mediated.

The results indicate that some PVN-spinal oxytocin neurones may selectively innervate cardiac sympathetic neurones. In view of a previous study (Yang *et al.* 2002) showing a significant influence of PVN-spinal vasopressin neurones on vasomotor neurones to the kidney we suggest there may be a functional cardiovascular coding represented by these two sets of peptidergic neurones.

Cechetto DF *et al.* (1988). *J Comp Neurol* **272**, 579–604.
Yang Z *et al.* (2002). *Exp Physiol* **87**, 663–674.
Yashpal K *et al.* (1987). *J Auton Nerv Syst* **20**, 167–178.

This work was supported by The British Heart Foundation

All procedures accord with current UK legislation

PC15

The role of prostaglandins (PGs) in the vasodilatation that follows isometric contraction in human subjects

Thet Su Win and Janice M. Marshall

Department of Physiology, The Medical School, Birmingham B15 2TT, UK

The issue of whether or not PGs contribute to the vasodilatation associated with muscle contraction is controversial (see Kilbom & Wennmalm, 1976; Shoemaker *et al.* 1996). In the present, double-blind cross-over study with approval of the University Ethics Review Committee, we tested the role of PGs in the dilatation that follows isometric contraction of the forearm.

In 11 healthy volunteers aged 21.5±0.8 years (mean ± S.E.M.), forearm blood flow (FBF) was measured by venous occlusion plethysmography and forearm cutaneous red cell flux (cRCF) by means of a laser Doppler probe. Measurement of arterial blood pressure (ABP) with a semiautomatic sphygmomanometer allowed calculation of forearm and cutaneous vascular conductance (FVC, CVC: FBF or cRCF divided by ABP).

In Protocol 1, measurements were made before and at 0, 0.5, 1, 2 and 3 min after a 2 min period of isometric contraction of the forearm at 60 % maximum voluntary contraction. This was performed after placebo or after aspirin (600 mg, a dose that blocks cyclooxygenase, Heavey *et al*, 1985). Following the contraction there was an increase in FVC (post-contraction dilatation), but no change in CVC, indicating the vasodilatation occurred predominantly in forearm muscle. Aspirin substantially reduced the post-contraction increase in FVC: from a baseline of 0.09±0.01 conductance units (CU), the peak FVC at 0 min was reduced from 0.24±0.03 to 0.14±0.01* CU (*:$P < 0.05$, placebo *vs.* aspirin, ANOVA and Bonferroni/Dunn post hoc test.

In Protocol 2, which was comparable to Protocol 1 except subjects breathed 40 % O_2 throughout, after placebo, the post contraction increase in FVC was smaller than during air breathing: the peak FVC at 0 min was 0.15±0.02* CU. Further, when breathing 40 % O_2, aspirin had no further effect on the post-contraction increase in FVC: the peak FVC at 0 min after aspirin and with 40 % O_2 was 0.16±0.02 CU.

These results indicate that PGs make a substantial contribution to the vasodilatation that follows isometric contraction of the forearm. Given aspirin and breathing 40 % O_2 had similar effects on the post-contraction dilatation, we propose the stimulus for PG synthesis is hypoxia of the vascular endothelium in forearm muscle that arises during the period of contraction.

Heavey DJ *et al.* (1985). *Nature* **318,**, 186–188.
Kilbom A & Wennmalm A, (1976). *J Physiol* **257**, 109–121.
Shoemaker JK *et al.* (1996) (1996). *J Appl Physiol* **81**, 1516–1521.

All procedures accord with current local guidelines and the Declaration of Helsinki

PC16

Dilator responsiveness to adenosine and the role of nitric oxide (NO) in the carotid circulation of young and mature rats under anaesthesia

Nisreen M. Omar and Janice M. Marshall

Department of Physiology, The Medical School, Birmingham B15 2TT, UK

We recently reported (Omar & Marshall, 2002) that the density of sympathetic innervation of middle cerebral and basilar arteries is substantially greater in mature than young Wistar rats. In the present study on young and mature male Wistar rats (~5, 12 weeks old respectively), we tested whether maturation affects vasodilator responses to adenosine.

In Study 1, carotid arteries (CA) were removed from rats under anaesthesia (3 % halothane in 3 l min^{-1} O_2). NO released from endothelial surface of the opened CA was measured directly using an NO sensitive electrode. In CAs from 6 young rats, any increase in NO output evoked by graded concentrations of adenosine (1×10^{-4}–5×10^{-3} M) was not dose-related (from 8.064 ± 2.814 to 8.847 ± 2.472 nM NO, mean ± S.E.M. with 1×10^{-4} and 5×10^{-3} M adenosine respectively). By contrast, in CAs from 7 mature rats, NO release evoked by adenosine was dose-related, ranging from 5.823 ± 1.153 to 16.126 ± 3.345*** nM NO (***; $P < 0.001$, ANOVA repeated measures) as described by Ray *et al*, (2002) for rat thoracic aorta.

In Study 2, on rats anaesthetised with Saffan (7–12 mg. kg^{-1}. h^{-1}), carotid blood flow (CBF) and carotid vascular conductance (CVC) were recorded during infusion of adenosine (2.4–2.8 mg kg^{-1} min^{-1} iv, for 3 min) to reduce arterial blood pressure (ABP) to ~60 mm Hg. In 7 young rats, adenosine evoked an increase in CVC (from 0.013 ± 0.001 to 0.033 ± 0.006††ml min^{-1} $mmHg^{-1}$; ††, †: $P < 0.01$, 0.05, Student's paired *t* test), but no change in CBF. By contrast, in 7 mature rats, a similar fall in ABP was accompanied by greater increase in CVC (from 0.019 ± 0.009 to 0.062 ± 0.003††ml min^{-1} $mmHg^{-1}$) and also by an increase in CBF (from 2.32 ± 0.44 to 3.71 ± 0.56††ml min^{-1}). In young rats, the NO synthesis inhibitor L-NAME (10 mg kg^{-1}) decreased baseline CVC to (0.009 ± 0.001†† ml min^{-1} $mmHg^{-1}$), but had no effect on the adenosine-evoked responses. However, in mature rats, L-NAME decreased baseline CVC to 0.007 ± 0.001†† ml min^{-1} $mmHg^{-1}$ and reduced the increase in both CVC and CBF induced by adenosine to 0.032 ± 0.006†† ml min^{-1} $mmHg^{-1}$ and 2.095 ± 0.39† ml min^{-1} respectively. At the end of experiments all animals were humanely killed.

These results suggest that the carotid circulation of young rats has a decreased dilator responsiveness to adenosine compared to mature rats and that may be explained, at least in part, by a lower NO output in response to adenosine in young rats.

Omar N & Marshall JM, (2003). *J Physiol* **547.P**, PC79.
Ray CJ *et al.* (2002). *J Physiol* **544**, 195–209.

All procedures accord with current UK legislation

PC17

Impact of dietary sodium on haeme-oxygenase distribution in rat kidneys

X.C. Wu and Edward J. Johns*

*Department of Physiology, The Medical School, Birmingham, UK and *Department of Physiology, University College Cork, Republic of Ireland*

The haemoxygenase enzyme isoforms (HO-1 and HO-2) generate carbon monoxide locally in vascular smooth muscle cells and may be important in regulating vessel tone and at this level may interact with nitric oxide (NO). There have been conflicting reports as to the contribution of the HO enzymes to salt-induced hypertension which has been hindered by the diversity in the duration and degree of dietary salt loading as well as to changing production of NO. The aim of the present study was to evaluate the effect of a short period of a modest elevation in dietary salt intake on HO-1 and HO-2 expression in different regions of the kidney with NO present and following its blockade and relate them to functional responses.

Male Wistar rats (0.3 ± 0.01 kg) were maintained on a normal rat diet but were given either tap water, saline (140 mmol NaCl), saline plus L-NAME (2.77 ± 0.7 mg/day) or water plus L-NAME to drink for a 7-day period. From day 4 to 7 fluid intake and output was estimated and creatinine clearance measured and used to calculate glomerular filtation rate (GFR). On day 7 the animals were anaesthetized (1 ml of chloralose/urethane, 16.5/250 mg, I.P.), blood pressure measured from a femoral artery, a blood sample collected and following an anaesthetic overdose to kill the rats, the kidneys were harvested. The kidneys were separated into cortex and medulla and HO-1 and HO-2 evaluated by Western blotting. Means ± S.E.M. were subjected to ANOVA and significance taken at $P < 0.05$.

Blood pressure in the normal rats ($n = 9$) was 103 ± 6 mmHg, but was increased by 24 % ($P < 0.01$) and 13 % in the rats maintained on saline plus L NAME and water plus NAME, respectively. GFR ($n = 7$), at 2.73 ± 0.38 ml min^{-1} kg^{-1} in the rats given water to drink, was similar in the rats given saline or L-NAME to drink but was decreased by 18 % ($P < 0.05$) in the rats on saline plus L-NAME. Analysis of the Western blots demonstrated that HO-2 was similar in both cortex and medulla and was not affected by any treatment. HO-1 was present to a higher degree in the cortex than medulla, giving a densitometric rato of 1.73 = 0.20 ($n = 10$). Drinking saline decreased the medullary expression of HO-1, such that the cortex to medulla ratio increased to 2.40 = 0.19 ($P < 0.05$) but L-NAME given to the animals receiving either saline or water, normalized the ratio (1.81 = 0.28 and 1.89 = 0.31, respectively).

These findings demonstrated that one week of saline intake depressed medullary but not cortical expression of HO-1 and that this appeared to be related and dependent upon the NO synthase activity. The exact way in which the HO-1 and NOS systems interact in response to the raised salt intake is unclear at present.

All procedures accord with current UK legislation

PC18

Connexin 45 in neurones of sensory, motor and autonomic pathways of murine brainstem and spinal cord

C.J. Milligan*, I.J. Edwards*, S. Maxeiner†, O. Kruger†, K. Willecke*, S.A. Deuchars* and J. Deuchars*

**School of Biomedical Sciences, University of Leeds, Leeds, UK and †Institut fur Genetik, Rheinische Friedrich-Wilhelms Universitat Bonn, D-53117, Bonn, Germany*

Electrical coupling typically mediated by gap junctions is evident between neurones such as sympathetic preganglionic neurones (e.g. Nolan *et al.* 1999) and motor neurones (Kiehn and Tresch, 2002). However, the gap junctional proteins involved remain to be identified. Here we investigate whether connexin45 (Cx45) is present in these neurones.

All animals were humanely killed by intraperitoneal injection of Sagatal (60 mg kg^{-1} I.P.) followed by transcardial perfusion with 0–4 % paraformaldehyde and 0–5 % glutaraldehyde in 0.1M phosphate buffer. Brainstem and spinal cord were sectioned on a vibrating microtome (50μm) and processed as indicated below. Expression of Cx45 was studied by localising expression of β-galactosidase in sections from 3 mice in which the entire coding region of the *Cx45* gene was replaced by the *lacZ* gene encoding for β-galactosidase (Kruger *et al.* 2000). For Cx45 immunohistochemistry 8 male Wistar rats (150–200g) and 3 C57Bl/6 mice (6–8 weeks) were perfused transcardially as above. In rats, autonomic preganglionic and motor neurones were pre-labelled by intraperitoneal injection of 0.1ml of 0.1 % of Fluorogold (Fluorochrome Int.) 3–7 days prior to perfusion. Sections were incubated in mouse or rabbit anti-Cx45 (1:100, Chemicon, U.K.) and these localized with appropriate Cy3 conjugated secondary antibodies raised in donkey (1:800, Jackson Immunochemicals).

Localisation of Cx45 (Cx45/lacZ) expression and immunoreactivity of Cx45 labelled the same populations of cells in mice and rats. Cx45 was strongly expressed in autonomic and motor nuclei throughout the brainstem and spinal cord, including the following nuclei: facial, abducens, dorsal vagal, ambiguus, hypoglossal and in the spinal cord the intermediolateral cell column and ventral horn. In these nuclei, Cx45 positive neurones also contained Fluorogold confirming that they were motor or preganglionic neurones. Cx45 in neurones that are constituents of sensory pathways included the cochlear, spinal trigeminal, dorsal column, inferior olivary nuclei as well as the area postrema and the dorsal horn.

These data suggest widespread expression of Cx45 in neurones in murine brainstem and spinal cord and are the first identification of a particular connexin protein in many of these neuronal populations.

Kiehn O & Tresch MC (2002). *Trends Neurosci* **25**, 108–115.

Kruger O *et al.* (2000). *Development* **127**, 4179–4193.

Nolan MF *et al.* (1999). *J Physiol* **519**, 753–764.

This work was funded by the Wellcome Trust (CJM), British Heart Foundation (SAD), the German Research Association (SM, KW) and a Physiological Society vacation studentship (IJE).

All procedures accord with current UK legislation