O1

Altered pattern of connectivity of the ventral respiratory group in adult monoamine oxidase A-deficient mice

S.P. Gaytan, G. Hilaire and R. Pásaro

Department of Physiology and Zoology, Faculty of Biology, University of Seville, Avda Reina Mercedes, 41102 Sevilla, Spain and Biology of Rhythm and Developement, GERM-CNRS, 280 Bd St Marguerite, 13009 Marseille, France

The high endogenous serotonin levels resulting from monoamine oxidase A (MAOA) deficiency in the Tg8 mouse (Cases *et al.* 1995) alter the prenatal maturation of the respiratory network and induce respiratory deficits in adults (Bou-Flores *et al.* 2000; Burnet *et al.* 2001). As the ventral respiratory group (VRG) contains structures necessary for respiratory rhythm generation, we wondered whether the VRG network was different in mutant Tg8 and normal albino Swiss strains (ASS).

Four adult male from both strains were deeply anaesthetised (chloral hydrate, 300 mg kg^{-1} I.P.). Their VRG was first localised by recording inspiratory multiunit activity with a glass micropipette (30–70 μm tip diameter, 4–8 MΩ) filled with a mixture of 2 % Fast Blue (FB, Sigma) and 10 % Fluororuby (FR, Molecular Probes). Then, pressure injections were made unilaterally at VRG sites (Picospritzer, General Valve). The animals were killed (under deep anaesthesia) 10 days after the injection. Histological sections were studied with a standardised procedure to evaluate quantitative differences (means ± S.E.M.; $P \leqslant 0.05$, Student's unpaired *t* test).

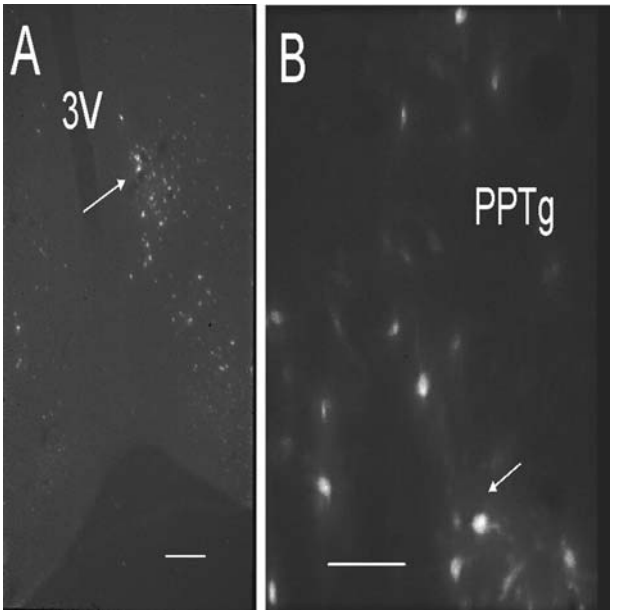

Figure 1. A and B, fluorescence photomicrographs (546 nm excitation wavelength) of blue fluorescent FB-labelled somata of ASS mouse. Bars, 200 μm; 3V, 3rd ventricle; PPTg, pedunculopontine tegmental nucleus. Arrows point to FB-labelled neurons.

FB-labelled neurons, FR-labelled fibres and terminal-like elements were found throughout the whole CNS (Tables 1 and 2) with inter-strain differences. First, the number of FB-labelled neurons was higher in the ASS than in the Tg8 in solitary tract, subcoeruleus, deep cerebellar, central periaqueductal grey (Figs 1 and 2), pedunculopontine tegmental (Fig. 1), deep mesencephalic, inferior colliculi, zona incerta, supraoptic and olfactory nuclei. In contrast, the number of FB-labelled neurons was higher in the Tg8 than in the ASS in obscurus and dorsal raphe nuclei, substantia nigra, posterior thalamic nucleus, and amygdalo-hippocampal area. In addition, the FR-labelled fibres showed an intense crossing at the level of raphe obscurus in Tg8 (Fig. 2) but not ASS mice. Finally, the FR-labelled varicosities were more abundant at the level of the cerebellum and inferior colliculi in ASS mice, but more abundant at the level of the amygdalo-hippocampal area in TG8 mice.

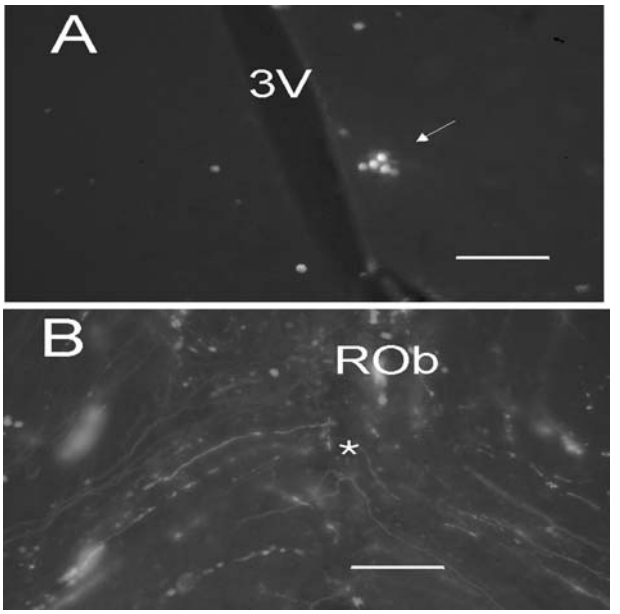

Figure 2. A, fluorescence photomicrographs (546 nm excitation wavelength) of blue fluorescent FB-labelled somata of Tg8 mouse. B, fluorescence photomicrographs (360 nm excitation wavelength) of red fluorescent FR-labelled fibres of Tg8 mouse. Bar, 200 μm; 3V, 3rd ventricle; ROb, raphe obscurus nucleus. Arrows point to a group of FB-labelled neurons. Asterisk indicates the mid-line.

Table 1. Summary of the main differences in the counts of FB-labelled neurons found within different CNS nuclei following the injection of the marker within the VRG in the Swiss and Tg8 mice

	Swiss		Tg8	
Labelled nuclei	i	c	i	c
Raphe obscurus	3		23	
Dorsal raphe	0		12	
Subcoeruleus	16 (10–1)	3 (2–1)	6 (4–1)	2 (2–0)
Periaq. central grey	249 (57–29)	91 (48–17)	45 (13–9)	30 (8–7)
Pedunculopont tegmental	56 (4–0)	32 (11–8)	6 (4–0)	(1–0)
Deep mesencephalic	102 (91–50)	21 (11–6)	2 (1–0)	1 (1–0)
Substantia nigra	4 (2–0)	2 (1–0)	18 (6–2)	12 (6–2)
Zona incerta	27 (14–0)	3 (1–0)	7 (5–0)	3 (2–0)
Supraoptic	20 (16–2)	0	3 (3–0)	0
Amig. hippocampal area	3(1–0)	1 (1–0)	23 (11–8)	1 (1–0)

i: ipsilatel, c: contraletral. The anterograde labelled fibres and terminal-like elements were counted in samples of 0.1 mm^2 per section. The data are totals and ranges.

Table 2. Summary of the main differences in the counts of FR-labelled fibres and terminal-like elements found within different CNS nuclei following the injection of the marker within the VRG in the Swiss and Tg8 mice

	Swiss		Tg8	
Labelled nuclei	i	c	i	c
Interposed cerebellar	+ + +	* * *	+ +	* *
Lateral cerebellar	+ + +	* * –	+ –	* –
Periaq. Central Grey	+ +	* *	+ +	*
Inferior colliculus	+ + +	* * *	+ +	* *
Lateral hypothalamic area	+ +	– –	– –	– –
Zona incerta	+ +	– –	– –	– –
Supraoptic	+ –	* –	– –	– –
Amig. Hippocampal area	– –	– –	+ –	* –

i: ipsilatel, c: contraletral. The anterograde labelled fibres and terminal-like elements were counted in samples of 0.1 mm^2 per section. –, no labelling; +/–, in some sections; +, 1–10 fibres; ++,11–25 fibres; +++, more than 25 fibres; */–, in some sections; *, 5–20 varicosities; **, 21–45 varicosities; ***, more than 45 varicosities.

In conclusion, the VRG is interconnected with different cerebellar, midbrain, diencephalic and telencephalic areas related to autonomic functions in both mouse strains, as well as in rats (Gaytán & Pásaro, 1998; Gaytán *et al.* 2002) but the adult VRG connectivity is altered by prenatal serotonin excess.

Bou-Flores C *et al.* (2000). *J Neurosci* **20**, 4646–4656.

Burnet H *et al.* (2001). *J Neurosci* **21**, 5212–5221.

Cases O *et al.* (1995). *Science* **268**, 1763–1766.

Gaytán SP & Pásaro R (1998). *Brain Res Bull* **47**, 625–642.

Gaytán SP *et al.* (2002). *Brain Res Bull* **57**, 335–339.

This work was supported by P.A.I. Junta de Andalucía and D.G.I. BFI2002-02055

All procedures accord with current National guidelines.

O2

Neonatal expression of 5-HT_{1B} receptors is altered by serotonin excess in monoamine oxidase A-deficient mice

S.P. Gaytan, J.C. Viemari, R. Pasaro and G. Hilaire

Department of Physiology and Zoology, Faculty of Biology, University of Seville, Avda Reina Mercedes, 41102 Sevilla, Spain and Biology of Rhythm and Developement, GERM-CNRS, 280 Bd St Marguerite, 13009 Marseille, France

Compelling evidence exists that prenatal serotonin (5-HT) plays a key role in CNS maturation. High endogenous levels of 5-HT resulting from monoamine oxidase A (MAOA) deficiency in the Tg8 transgenic strain of mice (created from the C3H/HeJ strain, C3H) alter the respiratory network maturation and the morphology of phrenic motoneurons (PhMns) (Cases *et al.* 1995; Bou-Flores *et al.* 2000). We compared neonatal C3H and Tg8 PhMns to know whether their 5-HT receptor expression was altered by prenatal 5-HT excess.

In ether-anaesthetised P0 neonates, brainstem and spinal cord were dissected, placed *in vitro* and the phrenic root was sucked within a rhodamine-filled suction electrode to label the PhrMns. Immunoreactivity of rhodamine-labelled PhMns was analysed by simple fluorescence and confocal microscopy with 5-HT_{2A} and 5-HT_{1B} receptor antibodies (Diasorin and Pharmigen, respectively).

In five C3H and seven Tg8 pups, respectively 38 and 32 PhMns showed red-stained and well-defined somata, primary and some distal dendrites. As reported (Bou-Flores *et al.* 2000), the number of primary dendrites was in the same range in both strains (4–6 per soma) but Tg8 distal dendrites showed frequent branching and varicosity-like profiles. 5-HT_{2A} immunoreactivity occurred in 24/38 C3H and 19/32 Tg8 somata, without obvious interstrain difference. The 5-HT_{1B} immunoreactivity was weak and diffusely distributed, without any relation to distinct cellular profiles in C3H whereas 14/32 Tg8 somata were clearly immunoreactive (Fig. 1). Finally, we analysed nine PhMns of C3H pups born from dams which received daily injections of the 5-HT_{2A} receptor agonist DOI (50 mg kg^{-1} day^{-1} from E16 to delivery) to activate the 5-HT_{2A} receptors of their fetuses. Two C3H PhMns expressed 5-HT_{1B} immunoreactivity similar to Tg8 PhMns.

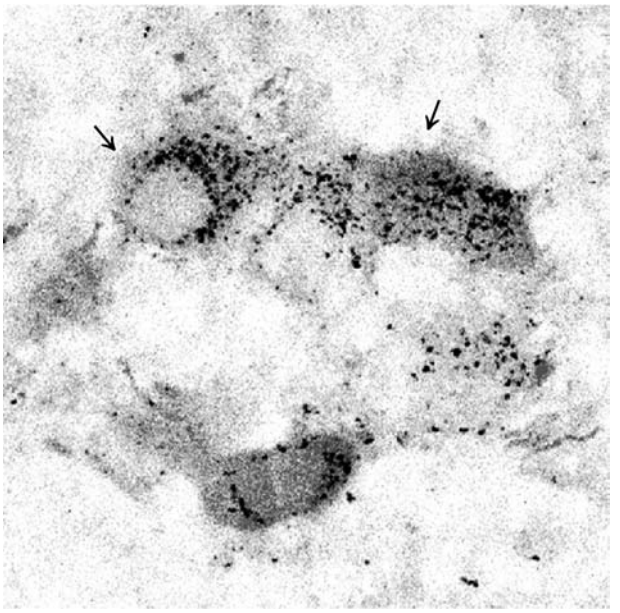

Figure 1. PhMns of Tg8 pups express 5-HT_{1B} immunoreactivity. Arrows show somata of rhodamine-labelled PhMns expressing black dots of 5-HT_{1B} immunoreactivity; this was never observed in C3H pups.

In conclusion, these results suggest that a high endogenous level of 5-HT induces 5-HT_{1B} receptor expression, probably through 5-HT_{2A} receptors, in neurons where they are normally not expressed.

Bou-Fores C *et al.* (2000). *J Neurosci* **20**, 4646–4656.

Cases O *et al.* (1995). *Science* **268**, 1763–1766.

This work was supported by P.A.I. Junta de Andalucía and D.G.I. BFI2002-02055.

All procedures accord with current National guidelines.

O3

Serotonergic activity in rat main cerebral arteries does not seem to be under GABAergic tone in dorsal raphe nucleus

E.J. Marco†, M.C. Fernández-Criado* and A.L. López de Pablo†

†*Departamento de Fisiología and* **Gabinete Veterinario, Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo, 2, 28029 Madrid, Spain*

Biochemical, pharmacological, and even morphological evidence supports that rat main cerebral arteries receive serotonergic fibres originating from dorsal raphe nucleus (DRN). The aim of the present work was to study whether these sertonergic fibres are under the influence of GABAergic innervation present in this nucleus. To achieve this, the effect of DRN injections of drugs related to the GABAergic system on arterial serotonergic activity was studied.

Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 130–180 g of the strain ICO:OFA SD (I.O.P.S. Caw) were used. The animals were housed in the proper facilities (rec. no. EX-021-U) complying with the European Community Directive 86/609/CEE and Spanish legislation (R.D. 223/1988) regarding the care of animals used in experimentation and other scientific purposes. The rats were anaesthetised (1 ml kg^{-1} of 4 % chloral hydrate, I.P.) and placed in a stereotaxic apparatus for DNR drug injection. Drugs were prepared in phosphate buffer solution of pH 7.2 and injected in a volume of 1 μl over 8–10 min. Control animals were submitted to the same procedure but received vehicle only. Afterwards, NSD-1015 (125 mg kg^{-1}, I.P.), an aromatic L-amino acid decarboxylase inhibitor, was administered and the rats killed by decapitation 1 h later. The brains were quickly removed on ice and the tissues (cerebral arteries, striatum and hippocampus) dissected out and placed on dry ice until their storage at −15 °C. Serotonergic activity was appraised from the accumulation of 5-hydroxytrytptophan (5-HTP) after decarboxylase inhibition. 5-HTP was assayed by reverse phase HPLC with electrochemical detection. Serotonergic activity in striatum was used as a control of the accuracy of the drug injection in DRN. Results are expressed as means ± S.E.M. in pmol per mg protein. Statistical analysis was performed using one-way ANOVA followed by a multiple comparison Bonferroni test or by Student's unpaired *t* test.

Local injection of a $GABA_A$ receptor agonist such as muscimol (0.1 μg) brought about a significant reduction in serotonergic activity in rat cerebral arteries when compared to control (1.20 ± 0.42, $n = 8$, and 4.28 ± 0.58, $n = 7$, respectively, $P < 0.01$) as well as in striatum (10.58 ± 0.68, $n = 8$, and, 14.68 ± 0.96, $n = 8$, respectively, $P < 0.05$). Previous local administration of 1 mg bicuculline, a $GABA_A$-receptor antagonist, avoided this reduction in both tissues (5.34 ± 0.77, $n = 9$, in cerebral arteries, and 17.04 ± 1.22, $n = 9$, in striatum). When baclofen (1 μg), a $GABA_B$ agonist, was injected in DRN, it also evoked a significant serotonergic activity decrease in cerebral arteries (2.16 ± 0.26, $n = 7$, $P < 0.05$) and striatum (10.85 ± 0.70, $n = 8$, $P < 0.001$), when compared to control (3.99 ± 0.40, $n = 6$, in cerebral arteries, and 13.58 ± 0.77, $n = 8$, in striatum). Previous local injection of a $GABA_B$-receptor antagonist, phaclofen (1 μg), could not antagonise this reduction (2.23 ± 0.14, $n = 7$, and 10.04 ± 0.74, $n = 7$, respectively). On the other hand, neither diazepam (1 μg), an agonist of the benzodiazepine subunit of the $GABA_A$ receptor, nor guvacine (1 μg), an inhibitor of GABA reuptake, significantly affected serotonergic activity in cerebral arteries and striatum when administered in DRN. The present results indicate that, although the cell bodies of the serotonergic fibres impinging on rat main cerebral arteries may possess GABAergic receptors, these do not seem to be submitted to a GABAergic tone. More experimental evidence needs to be produced in awake animals to eliminate the possibility that the absence of a direct GABAergic influence might be due to the fact that these experiments were undertaken in anaesthetised rats.

This work was supported by grant no. BF12000-0155 of Ministerio de Ciencia y Tecnología.

All procedures accord with current National guidelines.

O4

The parabrachial complex in the response to hypothalamic defence area stimulation in the rat: role of glutamate

M.S. Dawid-Milner, A. Díaz-Casares, M.V. López-González, J.P. Lara and S. González-Barón

Departamento de Fisiología Humana. Facultad de Medicina, Universidad de Málaga, 29080 Málaga, Spain

Hypothalamic defence area (HDA) activation produces a cardiorespiratory response characterised by tachypnoea with inspiratory facilitation, hypertension and tachycardia. The response is similar to that evoked during stimulation of lateral parabrachial nucleus (lPB) of the pons (Lara *et al.* 2002).

To characterise the role of glutamate in the cardiorespiratory response evoked by HDA stimulation, experiments were carried out in spontaneously breathing rats anaesthetised with sodium pentobarbitone (60 mg kg^{-1} I.P., supplemented as necessary with 20 mg kg^{-1} I.V.). At the end of the experiments animals were humanely killed.

The cardiorespiratory response evoked by electrical stimulation of the HDA (1 ms pulses, 20 μA, given at 100 Hz, over 5 s) was analysed before and after the microinjection of kynurenic acid (KA, 5 nmol), MK-801 (1 pmol) and CNQX (1 pmol) into both lPB and medial parabrachial nucleus (mPB) (50 nl over 5 s, pH 7, 4 ± 0.1 in phosphate-buffered saline). All data were compared statistically using Student's paired *t* test. Results are expressed as means ± S.E.M.

Inhibition of glutamate receptors with the microinjection of KA in lPB ($n = 7$) increased blood pressure and heart rate (from 105.1 ± 3.6 to 120.3 ± 2.8 mmHg, $P < 0.001$; from 339.8 ± 10.9 to 368.2 ± 10.2 b.p.m., $P > 0.001$); no changes were observed in respiratory rate. After KA microinjection the respiratory response to HDA stimulation was abolished; the pressure response and tachycardia were diminished (from 33.7 ± 10.9 to 6.8 ± 10.1 mmHg, $P < 0.001$; from 27.7 ± 10.4 to –0.05 ± 10.6 b.p.m., $P < 0.001$). KA was also microinjected in mPB ($n = 8$). After the injection of KA the pressure response and tachycardia to HDA stimulation were diminished (from 38.5 ± 7.6 to 16.7 ± 11.2 mmHg $P < 0.001$; from 27.4 ± 12 to –0.3 ± 14.2 b.p.m., $P > 0.001$) whilst no changes were observed in the respiratory response.

The inhibition of NMDA receptors with MK-801 ($n = 8$) or non-NMDA receptors with CNQX ($n = 8$) microinjected into both lPB and mPB increased arterial pressure and heart rate (lPB MK-801 from 108.4 ± 2.1 to 125.7 ± 2.4 mmHg, $P < 0.01$, and from 320.2 ± 14.5 to 371.9 ± 9.8 b.p.m., $P < 0.01$; lPB CNQX from 106.1 ± 1.6 to 118.5 ± 1.6 mmHg, $P < 0.01$, and from 352.4 ± 8.4 to 400.4 ± 12.8 b.p.m., $P < 0.01$); no changes were observed in respiratory frequency. MK-801 or CNQX within either lPB or mPB decreased the evoked tachycardia and the pressure response to HDA (PBl MK-801 from 41 ± 3.6 to 26.3 ± 5.3 mmHg $P < 0.001$, and from 36.9 ± 12.2 to 8.1 ± 13.4 b.p.m., $P < 0.01$; PBl CNQX from 40.3 ± 9.7 to 28.2 ± 10.3 mmHg, $P < 0.01$, and

from 36.4 ± 12.4 to –1.1 ± 13.8 b.p.m., $P < 0.001$; PBm MK-801 from 37.4 ± 11.8 to 26.5 ± 10.4 mmHg, $P < 0.05$; PBm CNQX from 39.2 ± 8.2 to 25.3 ± 10.2 mmHg, $P < 0.01$, and from 49.4 ± 9.6 to 31.1 ± 13.8 b.p.m., $P < 0.01$); no changes were observed in the intensity of the respiratory response.

These results suggest the importance of PB neurones modulating the cardiorespiratory response to HDA activation. Inhibition of lPB glutamate receptors (KA) abolished the classic tachypnoea evoked from HDA. The hypertension and tachycardia evoked from HDA were attenuated after specific inhibition of glutamate receptors (KA), NMDA receptors (MK-801) and non-NMDA receptors (CNQX) into either lPB or mPB neurones.

Lara JP *et al.* (2002). *Brain Res* **934**, 97–106.

This work was supported by the DGESIC PM99-0163, Spain.

All procedures accord with current National and local guidelines.

O5

Conflicting findings on the significance of voltage-dependent potassium channels in the transduction of hypoxia in the chemoreceptor cells of the carotid body

Angela Gomez-Niño, Ana Obeso, Jose Ramon Lopez-Lopez and Constancio Gonzalez

Departamento de Bioquímica y Biología Molecular y Fisiología-IBGM, Facultad de Medicina, Universidad de Valladolid-CSIC, 47005 Valladolid, Spain

The membrane model of hypoxic transduction in chemoreceptor cells of the carotid body (CB) considers that voltage-operated channels play an important role in evoking the release of neurotransmitters in response to hypoxia. To some authors, maxi-K^+ channels would contribute to control resting membrane potential in rat chemoreceptor cells (Wyatt *et al.* 1995), and thereby their hypoxic inhibition would depolarize the cells and trigger the release of neurotransmitters (Pardal *et al.* 2000); to some others maxi-K^+ would serve to control electrical events in depolarized cells, setting, but not triggering, the magnitude of the release response (Pepper *et al.* 1995; Buckler, 1997). There are some authors, however, who consider maxi-K^+ irrelevant to oxygen transduction (Donnelly, 1997; Lahiri *et al.* 1998). In rabbit chemoreceptor cells the available data imply that hypoxic inhibition of voltage-dependent transient potassium channels depolarizes the cells and triggers the hypoxic release response; or, in any case, it should be expected that voltage-dependent potassium channels would contribute to control electrical events in depolarized cells since in the hypoxic response in the rabbit cells tetrodotoxin-sensitive Na^+ channels participate (Gonzalez *et al.* 1994).

Intact rat and rabbit carotid bodies (CB) were isolated under pentobarbital anaesthesia (40–60 mg kg^{-1}, I.P.). Animals were killed with an overdose of the anaesthetic. To study the release of [^{3}H]catecholamines from chemoreceptor cells, CB catecholamine deposits were labelled by prior incubation of the organs with [^{3}H]tyrosine. We have measured the release while incubating the CB in normoxic (P_{O_2} ~150 mmHg), hypoxic (10 min; P_{O_2} ~46 and ~23 mmHg) high K^+ (10 min; 25 mM) and nicotine (10 min; 3×10^{-4} M)-containing solutions in the absence of drugs and in the presence of tetraethylammonium (a blocker of maxi-K^+ and transient K^+ currents and a nicotinic antagonist; 1–10 mM) and iberiotoxin (a specific blocker of maxi-K^+; 200 nM). In a few experiments we also used charybdotoxin (another maxi-K^+ blocker, 10–50 nM).

We have found that tetraethylammonium (5 mM) reduced markedly the release of [^{3}H]catecholamines induced by nicotine (97% in the rat and 93% in the rabbit), indicating that the blocker penetrates adequately in the intact CB. However, neither iberiotoxin nor tetraethylamonium altered the release of [^{3}H]catecholamines in normoxia, suggesting that the channels sensitive to the blockers do not participate in the setting of resting membrane potential of chemoreceptor cells. During hypoxic stimulation tetraethylamonium (5 mM) did not alter the release induced by hypoxia in the rat CB, but it augmented the hypoxic response in the rabbit CB by a 43% ($P < 0.05$), indicating that tetraethylamonium-sensitive K^+ channels play a role in setting of the magnitude of the hypoxic secretory response to hypoxia in the rabbit but not in the rat. The data suggest that in the rat tetraethylamonium-sensitive K^+ channels are not strictly required for the repolarization of chemoreceptor cells after hypoxic depolarization.

Buckler KJ (1997). *J Physiol* **498**, 649–662.
Donnelly DF (1997). *Respir Physiol* **110**, 211–218.
Gonzalez C *et al.* (1994). *Physiol Rev* **74**, 829-898.
Lahiri S *et al.* (1998). *Brain Res* **794**, 162–165.
Pardal R *et al.* (2000). *Proc Natl Acad Sci U S A* **97**, 2361–2366.
Pepper DR *et al.* (1995). *J Physiol* **487.P**, 177*P*–178*P*.
Wyatt CN *et al.* (1995). *Proc Natl Acad Sci U S A* **92**, 295–299.

This work was supported by Spanish DGICYT grants BFI2001-1713 and BFI2001-1691.

All procedures accord with current National guidelines.

O6

Intrinsic modifications of ventricular refractoriness by chronic exercise: a study in isolated rabbit heart

L. Such*, A. Alberola*, A. Rodríguez*, D. Sanmiguel, L. López†, P. Palau, B. Vilariño, J. Benavent, J. Ramírez, R. Pons*, C. García† and F.J. Chorro‡

**Department of Physiology,* †*Department of Physiotherapy and* ‡*Department of Medicine, University of Valencia, Valencia, Spain*

Modifications of electrophysiological properties of the myocardium induced by physical training have been classically attributed to the autonomic nervous system adaptation (Katona *et al.* 1982). We have previously reported that physical training decreases automatism and atrioventricular conduction by myocardial intrinsic mechanisms, which are not due to nervous and/or humoral influences (Such *et al.* 2002). Nevertheless, the results with respect to the refractoriness were less conclusive. We analyse in the present work the effects of a training programme on intrinsic ventricular refractoriness using an experimental model of isolated rabbit heart, and a modified methodology to explore ventricular refractoriness.

All the procedures were performed in accordance with the agreements of the European Convention of Strasbourg of March 18, 1986 (Instrument of ratification of 8/2/89, Official State Bulletin of 10/25/90). Eight NZW rabbits were submitted to a 6-week endurance exercise training programme, and six controls were not submitted to training. Three days after the chronic exercise programme was finished, rabbits were killed after anaesthesia with ketamine, and the heart excised and isolated in a Langendorff system. Extracellular electrodes were positioned for ventricular pacing and atrial electrograms, and a multiple electrode (121 electrodes) was connected to a

computerized epicardic mapping system of the cardiac electric activity (Map Tech) for ventricular electrograms. The following parameters were analysed: ventricle effective refractory period (VERP), and ventricle functional refractory period (VFRP). Refractory periods were analysed by means of extrastimulus test, and three basic ventricular stimuli trains (250, 200 and 150 ms) were employed. Parameters were compared between control and trained groups, and Student's unpaired *t* test was used. The results are given in Table 1.

Table 1. VERP and VFRP values in milliseconds calculated with basic trains of 250 ms, 200 ms and 150 ms

	250 ms		200 ms		150 ms	
	VERP	VFRP	VERP	VFRP	VERP	VFRP
Control	117±19 (5)	128±23 (5)	110±19 (5)	121±19 (6)	101±16 (6)	115±16 (6)
Trained	127±18 (8)	141±15 (8)	120±12 (8)	134±8 (7)	115±19 (6)	128±16 (6)

Data are means ± S.D.

Although no significant differences were observed, our results suggest a tendency towards an increase of intrinsic ventricular refractoriness by training.

Katona PG *et al.* (1982). *J Appl Physiol* **52**, 1652–1657.
Such L (2002). *J Appl Physiol* **92**, 225–229.

All procedures accord with current National guidelines.

O7

The effects of leptin on pressurised mesenteric artery and vein of the dog

Mohammed Jaffar*, Olusoga Sofola, David Myers, Mark Drinkhill and Roger Hainsworth

*Institute for Cardiovascular Research, University of Leeds, Leeds, UK and *Department of Physiology, Jordan University of Science and Technology, Jordan*

Leptin, a hormone produced by adipose tissue is associated with obesity as well as enhanced sympathetic nervous activity, hence its systemic administration results in elevated blood pressure whereas *in vitro* the hormone causes relaxation of the isolated aortic ring and this appears to be dependent on the production of endothelium-derived relaxing factor (EDRF-NO) (Vecchione *et al.* 2002). Since the pressurized technique is more physiological and since venous responses have not been assessed in response to leptin, the present experiments were designed to determine responses of the pressurized mesenteric resistance artery and vein of the dog to the hormone.

Beagle dogs weighing between 12 and 15 kg were anaesthetized with 100 mg kg^{-1} I.V. of α chloralose. The abdomen was opened and between occlusive ties, segments of the mesentery were removed and placed in ice-cold physiological salt solution (PSS) of the composition (mmol) 119 NaCl, 25 NaOH, 4.7 KCl, 1.6 $CaCl_2$, 1.17 $MgSO_4.7H_2O$, 1.18 KH_2PO_4, 0.026 NaEDTA and 5.5 glucose, following which the animal was killed by exsanguination. Third order mesenteric veins of diameter 900 μm as well as fourth order mesenteric arteries of diameter 400 μm were dissected out. The vessels were mounted between two glass cannulae in an arteriograph and pressurized to 70 mmHg in the case of the artery and to 10 mmHg in the case of the vein (Hainsworth *et al.* 2003), and were superfused with PSS of pH 7.4 warmed to 37°C and bubbled with 5 % CO_2–95 % O_2 gas mixture in a 100 ml reservoir. The vessels were allowed to equilibrate for 60–90 min, with two challenges of 10^{-6} M noradrenaline (NA) after which experiments were carried out.

Relaxation responses to leptin were determined in vessels that were pre-constricted with NA, before and after the administration of L-NAME (10^{-4} M), an inhibitor of EDRF-NO. In some of the vessels, responses of pre-constricted, L-NAME-administered vessels were also determined in response to 10^{-5} M sodium nitroprusside (SNP), an NO donor. In both vessels, the relaxation to leptin was dose dependent. In the artery ($n = 5$) at the concentration of 0.1, 1.0, 10.0 and 30.0 ng ml^{-1} the relaxation responses were (% ± S.E.M.) 4.3 ± 1.2, 9.6 ± 1.7, 15.5 ± 1.7 and 20.0 ± 1.6, respectively, and for the vein ($n = 8$) the corresponding values were 5.4 ± 0.7, 12.8 ± 0.5, 20.7 ± 0.9 and 28.3 ± 1.2. Following the administration of L-NAME, the responses of either the artery or the vein were virtually abolished, e.g. at the dose of 30 ng ml^{-1} of leptin, percentage relaxation responses were 4.2 ± 0.9 and 3.6 ± 0.5 in artery and vein, respectively. After L-NAME the relaxation response to SNP still occurred and in the artery this was 73.4 ± 6.8 % while in the vein it was 70.8 ± 7.4 %.

These results indicate that in the pressurized mesenteric artery or vein in the dog, leptin induces vascular relaxation which is mediated largely by EDRF-NO while the responses to the NO donor (SNP) were virtually preserved.

Hainsworth R *et al.* (2003). *Am J Hypertens* **16**, 6–10.
Vecchione C *et al.* (2002). *Diabetes* **51**, 168–173.

O.S. was a recipient of a Wellcome Trust travel award.

All procedures accord with current UK legislation.

O8

Simulated ventilation enhances systemic vascular responses to distension of the main pulmonary artery in anaesthetised dogs

J.P. Moore, R. Hainsworth and M.J. Drinkhill

Institute for Cardiovascular Research, University of Leeds, Leeds LS2 9JT, UK

Distension of the main pulmonary artery results in an increase in systemic vascular resistance (McMahon *et al.* 2000). However, significant vasoconstriction is not observed until the distension pressure is above 30 mmHg, which is higher than that normally observed in the pulmonary circulation. Recently we reported that afferent activity from pulmonary arterial baroreceptors is enhanced and that the 'set point' of this response is decreased when the chest is closed and phasic negative intrathoracic pressure is applied (Moore *et al.* 2002). The aim of this present study, therefore, was to re-examine the systemic vascular response to pulmonary artery distension, and to determine if this is altered by phasic intrathoracic pressure changes.

Eight dogs were anaesthetized with α chloralose (100 mg kg^{-1} I.V.) and artificially ventilated. The carotid sinuses were vascularly isolated and perfused at controlled pressures. A cardiopulmonary bypass was established that received blood from the left atrium and inferior vena cava, oxygenated it, and pumped it to a pressurized reservoir connected to cannulae inserted into the central and distal ends of the thoracic aorta. The subdiaphragmatic circulation was perfused at constant flow, and systemic perfusion pressure (SPP) provided an index of vascular resistance. A pouch consisting of the entire extrapulmonary parts of the pulmonary arteries and the trunk was created and this was independently perfused with venous blood from another pressurized reservoir. Strapping the ribs and sternum together

and then suturing the overlying muscle and skin tightly together resealed the chest cavity. The animals were humanely killed at the end of each experiment.

With intrathoracic pressure at atmospheric, the pulmonary arterial pressure (PAP) at the threshold for the response, which was taken to a 5% increase in SPP from baseline, was 31.2 ± 5.5 mmHg (mean ± S.E.M.). Simulation of physiological thoracic pressure changes by applying phasic negative intrathoracic pressure (minus 10 mmHg at 0.3 Hz) decreased the PAP at threshold to 21.2 ± 4.1 mmHg (*t* test, $P < 0.05$). The 'set point' of the stimulus–response curve was also significantly reduced, from 38.2 ± 4.5 mmHg to 25.5 ± 4..3 mmHg ($P < 0.05$).

We conclude that with physiological intrathoracic pressures, normal levels of pulmonary artery pressure induce reflex vasoconstriction and that pulmonary arterial baroreceptors are likely to have a role in circulatory control.

McMahon NC *et al.* (2000). *Exp Physiol* **85**, 411–420.
Moore JP *et al.* (2002). *J Physiol* **544.P**, 28*P*.

This work was supported by the British Heart Foundation.

All procedures accord with current UK legislation.

O9

Assessment of cardiorespiratory exercise function in obese children by body mass independent parameters

T. Reybrouck, J. Vinckx and M. Gewillig

Department of Cardiac Rehabilitation, Pediatrics and Pediatric Cardiology, University Hospital Gasthuisberg and Department of Rehabilitation Sciences, University of Leuven, 3000 Leuven Belgium

In obese patients submaximal brisk walking exercise may be very difficult to sustain, because of the extra metabolic burden imposed by the excess body mass. Parameters of maximal aerobic exercise performance may be strikingly reduced when expressed per kg body mass. The aim of the present study was to analyse whether cardiorespiratory exercise function is truly impaired in obese children and adolescents, when parameters of aerobic exercise function are used which are independent of body mass. Therefore the kinetics of oxygen uptake ($\dot{V}_{O_2}$) at the onset of exercise were studied by analysis of the normalised oxygen deficit.

The patients underwent square wave exercise testing on a treadmill. The speed was set at 5 km h^{-1} and the inclincation at 4%. The oxygen deficit was calculated by subtracting the $\dot{V}_{O_2}$ measured at the onset of exercise from the steady-state $\dot{V}_{O_2}$ obtained at the end of the exercise. These differences were cumulated and expressed as a percentage of the cumulated oxygen cost for the 6 min exercise test. All data are expressed as means and standard deviation. Differences between groups were calculated by Student's unpaired *t* test. The local medical ethical committee approved the study. The subjects were 17 obese patients (mean age: 11.2 ± 2.6 years), body mass was 70.7 ± 21.4 kg, body mass index averaged 28.9 ± 2.8 and the percentage overweight was 53.9 ± 16.5%. The patients were compared to a group of 18 normal controls of comparable age: 11.6 ± 2.2 years ($P > 0.25$ patients *vs.*controls), body mass was 40.4 ± 10.6 kg ($P < 0.001$, patients *vs.* controls).

In the obese patients, the oxygen deficit amounted to 7.2 ± 1.9% and was not significantly ($P > 0.25$) different from the value obtained in normal controls: 6.9 ± 1.0%. However obese patients exercised at a higher percentage of the maximal heart rate (79% in the obese subjects *vs.* 70% for normal controls). Due to a less efficient walking economy during treadmill exercise, $\dot{V}_{O_2}$ (expressed per kg body mass) during submaximal exercise was slightly higher in the obese (22.3 ± 2.7 ml O_2 min^{-1} kg^{-1}) compared to the normal controls (20.2 ± 2.4 ml O_2 min^{-1} kg^{-1}) ($P < 0.05$).

The similar values for O_2 deficit at the onset of exercise in obese patients compared to normal controls shows that there is no evidence of a cardiovascular limitation of exercise capacity in obese patients. Due to a less efficient walking pattern, $\dot{V}_{O_2}$ during submaximal exercise was higher in the obese patients. Therefore a same absolute work intensity is perceived as more strenous in obese subjects compared to normal controls.

This study was supported in part by the Foundation for Research in Pediatric Cardiology.

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

O9b

Organisation of projections from the central nucleus of amygdala to autonomic neurones in the medulla: a light and electron microscopic study

Sikha Saha, Eric K.A. Corbett, J.P. Moore, Mark J. Drinkhill and Trevor F.C. Batten

Institute for Cardiovascular Research, University of Leeds, Leeds LS2 9JT, UK

The central nucleus of amygdala (CeA), a limbic forebrain structure, has an important role in the integration of emotional and cardiovascular responses (Davis, 1992). Recently, we have provided anatomical evidence for an inhibitory neural pathway by which cardiovascular responses to stress may be, in part, mediated by attenuation of baroreceptor reflexes at the level of the NTS (Saha *et al.* 2000, 2002). The present study was designed to investigate the organisation of projections from the CeA to neurones in the dorsal vagal motor nucleus (DVN), the nucleus ambiguus (NA) and the rostral ventrolateral medulla (RVLM). The first two nuclei contain cardioinhibitory vagal preganglionic neurones and the RVLM contains tonically active neurons that project to preganglionic sympathetic neurons in the spinal cord.

All recovery experiments were performed on 280–300 g rats ($n = 12$) anaesthetised with halothane (95% in O_2) with buprenorphine analgesia as required. The CeA terminals in the medulla were labelled by microinjecting an anterograde tracer, biotin dextran amine (BDA, 0.2 μl of 10% in saline) stereotaxically into the CeA. Neurones in the DVN and NA were identified by applying retrograde tracer, cholera toxin B subunit (CTb, 0.5–1 μl of 1% in saline) into the nodose ganglion, and neurones in the RVLM were identified by phenylethanolamine N-methyl-transferase (PNMT) immunocytochemistry or by expression of the immediate early gene c-fos following infusion of sodium nitroprusside (1 mg ml^{-1} in saline for 1 h under pentobarbitone anaesthesia), at a rate sufficient to maintain mean arterial pressure at least 25% below the resting level. At the end of the experimental protocol, the rats were humanely killed by perfusion with aldehyde fixative, and vibratome sections of the brain stem were processed for light and electron microscopy.

BDA labelled CeA terminals were found to make synaptic contact with neurones retrogradely labelled with CTb in the DVN and NA, and also with PNMT-positive neurones in the RVLM. NP-induced hypotension produced c-fos expression in the RVLM. Many anterogradely labelled varicose fibres were observed in close apposition with neurones with Fos-immunoreactive nuclei in the RVLM. The results suggest that neural projections from

the CeA may directly influence the activity of medullary neurones, including sympathetic premotor neurones and parasympathetic preganglionic neurones that are involved in regulation of blood pressure and other autonomic functions in response to stressful conditions.

Davis M (1992). *Annu Rev Neuroscience* **15**, 353–375.
Saha S *et al.* (2000). *Neuroscience* **99**, 613–626.
Saha S *et al.* (2002). *J Physiology* **544.P**, 29*P*.

This work was supported by the Wellcome Trust.

All procedures accord with current UK legislation.

P79

Involvement of oxytocin in central cardiovascular regulation. Possible modulation of baroreceptor reflex

Carmen Vela, Sandra Pérez, Zaida Díaz-Cabiale, Rosa Garrido, Rafael Coveñas, Salvador González-Barón and José Angel Narváez

Depto de Fisiología, Facultad de Medicina, Universidad de Málaga, Spain

Oxytocin (OXT) is a peptide involved in diverse physiological functions in the central nervous system including central cardiovascular regulation. Previously it has been demonstrated that OXT microinjected in the nucleus tractus solitarii (NTS) elicits a dose-dependent increase of mean arterial pressure (MAP) and heart rate (HR). Moreover, OXT blocks the vasodepressor responses induced by α_2-adrenergic agonists and decreases α_2-adrenergic receptor binding within the NTS. The aim of this work was to investigate if the cardiovascular actions of OXT were mediated through specific OXT receptors and if OXT could modulate the cardiovascular responses elicited by L-glutamate, the main transmitter of the primary baroreceptor afferents.

Experiments were performed in urethane-anaesthetised rats, and animals received unilateral injection in the NTS in a total volume of 50 nl. Mean arterial pressure (MAP) and HR were recorded from the femoral artery over 30 min. To determine if the action of OXT is mediated by specific receptors, animals received microinjections of OXT (10 pmol) alone or together with the OXT-selective antagonist receptor $d(CH_2)_5$,$Tyr(Me)_2$,Orn_8-vasotocin (OXA) (10 pmol). These results were analysed using one-way ANOVA followed by Fisher's post test. To test the possible modulation of OXT on the cardiovascular response induced by L-glutamate (L-Glu) other groups of rats received coinjections of a threshold dose of OXT (1 pmol) plus an ED_{50} dose of L-Glu (1.5 nmol), or with threshold doses of both OXT (1 pmol) and L-Glu (30 pmol). These results were analysed using Student's unpaired *t* test. Control rats received CSF alone. At the end of the experiments, the rats were humanely killed.

The presence of OXA blocked the vasopressor response of OXT. This blockade was partial ($P < 0.05$) in the first 15 min of recording but complete ($P < 0.001$) when it was analysed for the 30 min recording period. However, OXA significantly blocked the tachycardic response of OXT ($P < 0.01$) immediately after coinjections. With regard to the modulation of L-Glu responses, threshold doses of OXT significantly blocked ($P < 0.001$) the vasodepressor responses elicited by the ED_{50} dose of L-Glu by 80 %, without any modification of its bradycardic effect. Coinjections of threshold doses of OXT and L-Glu did not produce any change in MAP values, but elicited a bradycardia.

These results demonstrate that cardiovascular actions of OXT in the NTS are mediated by specific OXT receptors and suggest an involvement of OXT on the modulation of baroreceptor reflex within the NTS.

This work has been supported by the Spanish CICYT (BFI2001-1905).

All procedures accord with current National guidelines.

P80

Interactions between the hypothalamic defence area and the A5 region: role of glutamate

M.V. López-González, M.S. Dawid-Milner, A. Díaz-Casares, C.A. Peinado, J.P. Lara and S. González-Barón

Departamento de Fisiología Humana, Facultad de Medicina, Universidad de Málaga, 29080 Málaga, Spain

Stimulation of the hypothalamic defence area (HDA) in rats produces a cardiorespiratory response characterised by an increase of respiratory rate, tachycardia and a marked pressor response. The cardiovascular response is similar to that obtained during chemical stimulation of the A5 catecholaminergic region of the pons. We have demonstrated a functional interaction between these two regions inhibiting A5 neurones with muscimol (Dawid-Milner *et al.* 2001).

In order to characterise the role of glutamate in the cardiorespiratory response to HDA stimulation, experiments were carried out in spontaneously breathing anaesthetised rats (sodium pentobarbitone 60 mg kg^{-1} I.P., supplemented with 20 mg kg^{-1} I.V.). At the end of the experiments animals were humanely killed.

The cardiorespiratory response evoked by electrical stimulation of the HDA (1 ms pulses, 20–50 μA, given at 100 Hz, for 5 s) was analysed before and after the microinjection of kinurenic acid (5–10 nmol), MK-801 (1 pmol), MCPG (0.1 nmol) and CNQX (1 pmol) into the A5 region (50 nl, 5 s, in PBS pH 7.4 ± 0.1). All data were compared statistically using Student's paired *t* test. Results are expressed as mean ± S.E.M.

The inhibition of glutamate receptors with the microinjection of kinurenic acid ($n = 9$) into the A5 region increased resting mean arterial pressure ($P < 0.01$). No changes were observed in resting respiratory frequency or heart rate. After the microinjection of kinurenic acid the pressor response and the tachycardia to HDA stimulation were diminished (36.7 ± 3.2 to 16.8 ± 2.6 mmHg $P < 0.001$; from 35.9 ± 4.4 to 11.2 ± 5.4 b.p.m., $P < 0.001$). No change in the respiratory response was observed.

No changes of cardiorespiratory parameters at rest were observed after the inhibition of NMDA receptors with the microinjection of MK-801 ($n = 8$) into the A5 region. No changes were observed in the cardiorespiratory response to HDA stimulation after the microinjection of MK-801 in A5 region.

The inhibition of non-NMDA receptors with CNQX ($n = 8$), or metabotropic receptors with MCPG ($n = 8$) increased resting mean arterial pressure (CNQX 105.1 ± 0.93 to 113.2 ± 2.5 mmHg, $P < 0{,}01$; MCPG from 104.2 ± 1.1 to 119.1 ± 1.6 mmHg, $P < 0.01$). No changes were observed in resting respiratory frequency or heart rate. The microinjection of CNQX or MCPG within the A5 region decreased the evoked tachycardia (CNQX from 29.7 ± 6.6 to –8.3 ± 10.6 b.p.m., $P < 0.001$; MCPG from 29.5 ± 4.3 to 11.5 ± 3.7 b.p.m., $P < 0.01$) to HDA stimulation. No changes were observed in the intensity of the pressor or tachypnoeic response.

These results suggest that the inhibition of A5 glutamate receptors decreases the tachycardia evoked on HDA stimulation.

New data on the role of the A5 region in the control mechanisms of cardiorespiratory activity are also inferred.

Dawid-Milner MS *et al.* (2001). *Pflügers Arch* **441**, 434–443.

This work was supported by the DGESIC PM99-0163, Spain.

All procedures accord with current National and local guidelines.

P81

The influence of the PVN-spinal projection on renal sympathetic activity after blockade of the vasomotor area in the rostral ventrolateral medulla in Wistar rats

Zhuo Yang and John H. Coote

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

Some hypothalamic paraventricular (PVN) parvocellular neurones either project to spinally projecting neurones in the rostral ventrolateral medulla (RVLM) or to sympathetic preganglionic neurones in the spinal cord or to both (Pyner & Coote, 2000). In the present study the functional influence of the PVN-spinal projection was tested by examining blood pressure (BP) and renal sympathetic nerve activity (RSNA) responses to PVN stimulation (D,L-homocysteic acid, DLH 0.2 M) in the presence and absence of RVLM-spinal influences.

Fourteen Wistar rats were anaesthetised with urethane and chloralose and a glass micropipette was inserted into PVN for microinjection of DLH. A double-barrelled glass micropipette was placed into the area of RVLM for microinjection of drugs. Change in the efficacy of spinal pathways was tested by intrathecal (I.T.) application of drugs via a catheter inserted via the foramen magnum so that its tip lay at *T*10. Statistical analysis was performed using Student's two-tailed, paired *t* test. Rats were killed by overdose of urethane anaesthetic at the end of experiment.

After identifying an excitatory site in the PVN on the left side, a micropipette was stereotaxically positioned in the ventral medulla on both sides and the rostral ventrolateral vasomotor area identified by microinjection of glutamate (1 nmol). A number of control BP and RSNA responses were then recorded following which the GABA agonist muscimol (200 pmol) was microinjected into both left and right RVLM. Muscimol caused a small fall in BP from 72.4 ± 3.7 mmHg to 68.8 ± 3.4 mmHg (means ± S.D.; NS); reduced RSNA from 13.3 ± 1.6 Hz to 11.8 ± 1.7 Hz, $P < 0.002$) and abolished a baroreflex inhibition of RSNA induced by pressor response to phenylephrine I.V. (4–6 μg). However bilateral muscimol into RVLM did not reduce the BP increase in response to PVN stimulation (9.2 ± 1.1 mmHg before, 12.6 ± 2.5 mmHg after) or the increase in RSNA (69.4 ± 13.1 % before, 62.9 ± 13.9 % after). Intrathecal application of the glutamate antagonist kynurenic acid (10 μl, 4 mM, Sigma) after muscimol block of RVLM, reduced the PVN-BP response from 14.7 ± 3.1 mmHg to 7 ± 1.4 mmHg ($P < 0.07$) and the PVN-RSNA response from 55.9 ± 13.5 % to 35.8 ± 12.1 % ($P \leqslant 0.05$). The PVN sympathoexcitation were also reduced by I.T. V1a antagonist (10 μl, 0.05 mM, Sigma), BP from 18.9 ± 3.8 mmHg to 12.9 ± 2.4 mmHg ($P \leqslant 0.2$) and RSNA from 48.6 ± 10 % to 38.5 ± 8.8 % ($P = 0.1$).

The results indicate that PVN-spinal neurones can act independently of RVL to increase sympathetic vasomotor activity and that glutamate or vasopressin neurones may contribute to this effect.

Pyner S & Coote JH (2000). *Neuroscience* **100**, 549–556.

This work was funded by The Wellcome Trust.

All procedures accord with current UK legislation.

P82

Effect of AT1 receptor blockade on hepatic redox status in spontaneously hypertensive rats: possible relevance for endothelial function

Eva Cediel*, David Sanz-Rosa*, M. Pilar Oubiña*, Natalia de las Heras*, Africa González*, Onofre Vegazo†, Javier Jiménez†, Vicente Lahera* and Victoria Cachofeiro*

**Department of Physiology, School of Medicine, Universidad Complutense, Madrid and †Medical Department, AstraZeneca Farmacéutica Spain, S.A., Spain*

The aim of this study was to evaluate whether the amelioration of endothelial dysfunction with candesartan, an AT_1 angiotensin II antagonist, in spontaneously hypertensive rats (SHR) was associated with modification of the hepatic redox system.

SHR ($n = 18$; 22 weeks old) were treated or not with candesartan (2 mg kg^{-1} day^{-1}) for 8 weeks. Wistar Kyoto rats (WKY; $n = 8$) of the same age were used as a normotensive reference group. At the end of the treatment period, systolic arterial pressure (SAP) was measured. Rats were killed by decapitation, and vascular reactivity to acetylcholine (ACh; 10^{-10}–10^{-7} M), sodium nitroprusside (SNP; 10^{-10}–10^{-7} M) and acetylcholine + L-NAME (ACh + L-NAME; 10^{-8}–10^{-5} M) was studied in aortic rings. Hepatic levels of malonyl dialdehyde (MDA), GSH/GSSG ratio, glutathione peroxidase (GSHPx) and glutathione reductase (GSHRed) were measured in liver homogenates using specific assays. Aortic eNOS expression was measured with Northern blot analysis. All the experiments were carried out following recommendations from the institutional animal care and use committee, according to the guidelines for ethical care of experimental animals of the European Union. Dose–response curves were compared by multivariate analysis of variance for repeated measures (MANOVA) using the SPSS 10.0 program. All other data were analysed using a one-way analysis of variance, followed by a Newman-Keuls test if differences were noted. The null hypothesis was rejected when the *P* value was less than 0.05.

SAP was higher ($P < 0.05$) in SHR than in WKY and was reduced ($P < 0.05$) by candesartan. ACh relaxations were smaller ($P < 0.05$) and contractions induced by ACh + L-NAME were greater ($P < 0.05$) in SHR than in WKY. Treatment with candesartan enhanced ($P < 0.05$) ACh relaxations and reduced ($P < 0.05$) endothelium-dependent contractions. MDA levels were higher ($P < 0.05$) and GSH/GSSG ratio and GSHPx were lower ($P < 0.05$) in SHR than WKY. Candesartan reduced ($P < 0.05$) MDA and increased ($P < 0.05$) GSH/GSSG ratio without affecting either GSHPx or GSHRed. Expression of eNOS mRNA was similar in WKY and SHR, and candesartan increased ($P < 0.05$) it in SHR

Amelioration of hepatic antioxidant defence, as well as enhancement of eNOS gene expression and reduction of endothelium-dependent contractions, produced by candesartan could contribute to improve endothelial dysfunction in SHR. The results further support the role of AII in the functional vascular alterations produced by hypertension.

All procedures accord with current National and local guidelines.

P83

Inhibition of 5-hydroxytryptamine-mediated contraction by α_1-adrenoceptor antagonist in mouse thoracic aorta

Z. Ali*, J.C. McGrath*, G. Tsujimoto† and C.J. Daly*

**Division of Neuroscience & Biomedical Systems, IBLS, University of Glasgow, Glasgow, UK, G12 8QQ and †Department of Molecular, Cell Pharmacology, National Childrens Medical Research Center, Tokyo, Japan*

Mouse thoracic aorta contracts to 5-hydroxytryptamine through mainly 5-HT_{2A} receptors (McKune & Watts 2001). Shaw *et al.* (2000) demonstrated antagonism of 5-HT responses by prazosin (α_1 selective) in rat pulmonary arteries suggesting 5-HT was acting at α_1-adrenoceptors (ARs). The adrenergic response in the aorta has been subtyped as the α_{1D}-AR (Yamamoto & Koike, 2001; Daly *et al.* 2002). We have now investigated the adrenergic action of 5-HT in the aorta using a transgenic approach.

Four-month-old male (30–40 g) 129/Sv/C57BL/6J control (WT, $n = 5$) and mice lacking α_{1D}-ARs (Tanoue *et al.* 2002) (α_{1D}-KO, $n = 7$) were killed by CO_2 and their aortae isolated. Rings (2 mm) were mounted on a wire myograph in Krebs solution at 37°C. After initial challenges to 125 mM KCl and 10 μM phenylephrine cumulative concentration response curves (1 nM–300 μM) were constructed to 5-HT in the presence or absence of prazosin (10 nM). EC_{50} and EC_{25} values were compared using a one-way-ANOVA with a Bonferonni post test.

Table 1. pEC_{25} and pEC_{50} values of 5-HT ± 10 nM prazosin in both WT and α_{1D}-KO mice

	pEC_{25}	pEC_{50}
WT (Control)	7.53 ± 0.13	7.09 ± 0.09
WT ($+10^{-8}$ M Prz)	6.94 ± 0.08**	6.69 ± 0.07*
α_{1D}-KO (Control)	7.33 ± 0.09	7.05 ± 0.07
α_{1D}-KO ($+10^{-8}$ M Prz)	7.38 ± 0.09	7.08 ± 0.07

Data are means ± S.E.M. Statistical analysis performed within same strain against control (*$P < 0.05$, ** $P < 0.01$).

There was no significant difference in maximum responses across all four groups. Sensitivity of 5-HT responses of the WT and α_{1D}-KO mice were similar (Table 1). In WT mice 10 nM prazosin shifted the EC_{50} (dose ratio = 2.51) response but the shift was more pronounced at the EC_{25} (dose ratio = 3.89). In the α_{1D}-KO the 5-HT response was unaltered by the presence of 10 nM prazosin.

Our results agree with Shaw *et al.* (2000) that 5-HT is sensitive to prazosin, shown by the EC_{50} shift in the WT. However instead of a parallel shift we saw a greater shift by prazosin at the EC_{25}. The antagonism by prazosin of 5-HT does not occur in the α_{1D}-KO. Interestingly the loss of the α_{1D}-AR has not affected the sensitivity of the aorta to 5-HT. This suggests there may be compensation by 5-HT receptors to accommodate for the response lost with the knockout of the α_{1D}-AR. In conclusion, we have shown that the action of 5-HT is partly mediated by α_{1D}-ARs in WT mouse aorta.

Daly *et al* (2002). *Physiol Genomics* **9**, 85–91.

McKune & Watts (2001). *J Pharmacol Exp Ther* **297** , 88–95.

Shaw *et al* (2000). *Pulmonary Pharmacol Ther* **13**, 277–285.

Tanoue *et al* (2002). *J Clin Invest* **109**, 765–755.

Yamamoto & Koike (2001). *Eur J Pharmacol* **424** , 131–140.

This research was supported by MRC. The group is a member of the EC FP5 project 'Vascan' (QLG1-CT-1999-00084).

All procedures accord with current UK legislation.

P84

Removal of the α_{1B}-adrenoceptor uncovers an α_{1D} component in mesenteric resistance arteries

M. McBride*, C.J. Daly*, G. Tsujimoto† and J.C. McGrath*

**Division of Neuroscience & Biomedical systems, IBLS, University of Glasgow, Glasgow G12 8QQ, UK and †Department of Molecular, Cell Pharmacology, National Childrens Medical Research Center, Tokyo, Japan*

α_{1A}-Adrenoceptors play the dominant vasoconstrictor role in mouse mesenteric resistance arteries (Daly *et al.* 2002). We now analyse the receptor population remaining after removal of the α_{1B} or α_{1D}-adrenoceptor (Tanoue *et al.* 2002) by receptor knockouts (KO).

Sixteen-month-old male (22–31 g) (C57Bl(WT), α_{1B}KO, α_{1D}KO) mice were killed by CO_2 and mesenteric arteries isolated. Rings (2 mm) were mounted in Krebs at 37°C on a wire myograph. Cumulative phenylephrine (α_1 agonist) was administered alone or in the presence of 5-methylurapadil (5MeU) (α_{1A}-selective antagonist; 1×10^{-7} M) or BMY7378 (α_{1D}-selective antagonist; 1×10^{-7} M), concentrations chosen for subtype selectivity.

α_{1B} and α_{1D}KOs had greater maxima than controls. α_{1D} knockout had reduced sensitivity to phenylephrine compared to WT and α_{1B}KO (Table 1). 5MeU caused a rightward shift in all three strains. However in the α_{1B}KO the response became biphasic (Fig. 1). This second component is abolished by BMY7378, indicating the α_{1D}-adrenoceptor. BMY7378 failed to shift the response further in either the α_{1D}KO or in WT.

Table 1. Contractile max. (g force) and pEC_{50}, pEC_{20} and dose ratios at pEC_{20} for WT, α_{1B} and α_{1D}

	WT	α_{1B}	α_{1D}
1st curve max	0.31 ± 0.02	0.54 ± 0.07	0.61 ± 0.06
1st curve pEC_{50}	5.7 ± 0.02	5.7 ± 0.02	5.3 ± 0.02
pEC_{20} with 5MeU	4.9 ± 0.12	4.9 ± 0.38	4.1 ± 0.09
Dose ratio			
5MeU/BMY7378	106.5 ± 54.0	110.1 ± 56.36	106.0 ± 14.0

Data are means ± S.E.M., $n = 7$ for all strains.

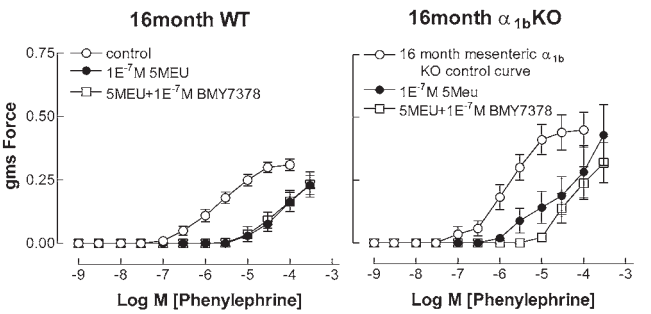

Figure 1. PE-induced contractions in WT and α_{1B}KO.

Removal of either of the 'minor' α_1-adrenoceptor subtypes (α_{1B} or α_{1D}) affects the contractile function of mesenteric resistance arteries. In the absence of α_{1D} receptors, the maximum is greater (unexplained) but sensitivity to phenylephrine is decreased (suggesting an α_{1D} response in WT). Absence of α_{1B}-adrenoceptor increases sensitivity to α_{1D}; a 5MeU-resistant, BMY7378-sensitive component appears. This illustrates that removal of one α_1 subtype can alter the functional response via the remaining receptor population. The presence of multiple α_1 subtypes that has dogged pharmacological analysis is confirmed. The α_{1D} subtype, dominant in conducting arteries, contributes a

minor component in these small 'resistance' arteries that might be upregulated by modulatory or pathophysiological factors.

Daly *et al.* (2002). *Physiol Genomics* **9**, 85–91.
Tanoue *et al.* (2002). *J Clin Invest* **109**, 765–755.

M.McB. is supported by the British Heart Foundation. Our group is a member of the EC FP5 project 'Vascan' (QLG1-CT-1999-00084).

All procedures accord with current UK legislation.

P85

Carotid baroreflex function during upper body exercise in humans

S. Volianitis, S. Ogoh*, W. Wray*, X. Shi*, P.B. Raven* and N.H. Secher

*The Copenhagen Muscle Research Centre, Department of Anaesthesia, Rigshospitalet, University of Copenhagen, Denmark and *Department of Integrative Physiology, University of North Texas, Fort Worth, TX, USA*

The operating point of the carotid baroreflex (CBR) resets during exercise in direct relation to the intensity and the recruited muscle mass (Norton *et al.* 1999). However, during arm cranking (A) blood pressure is higher than during combined arm and leg exercise (A+L) (Volianitis & Secher, 2002). This study evaluated the carotid baroreflex (CBR) function during leg cycling (L), A, and A+L.

CBR stimulus–response relationships were compared in 12 volunteers by using the neck pressure–neck suction technique during dynamic exercise. Exercise intensities for A and L were chosen to elicit a heart rate response of ~100 and ~120 b.p.m., respectively. A+L was performed with the combination of the same absolute workloads used in the A and L trials. Comparisons across exercise conditions were made with one-way ANOVA with repeated measures and Student-Newman-Keuls t test. Significance was accepted at $P < 0.05$.

The position of the operating point (the prestimulus blood pressure) of the carotid–vasomotor reflex at rest was 87.9 ± 5.6 mmHg (mean ± S.E.M.) and during L, A+L and A it was 91.4 ± 6.9, 103.1 ± 7.2 and 109.4 ± 9.3 mmHg, respectively ($P < 0.05$). The threshold and saturation pressures of the reflex during A were >A+L, >L and >than at rest ($P < 0.05$) with no significant change in the maximal reflex gain.

These findings suggest the CBR during combined arm and leg exercise resets lower than during arm cranking alone.

Norton KH *et al.* (1999). *J Appl Physiol* **87**, 332–8.
Volianitis S & Secher NH (2002). *J Physiol* **544**, 977–984.

S.V. was supported by a Marie Curie Fellowship of the European Community programme Improving Human Potential under contract number HPMF-CT-2000-00526.

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

P86

Evidence of interdependence between central nervous and respiratory activity in healthy human term neonates during sleep

Soledad Mañas*, José M. Garrido*, Julián J. González†, Luis De Vera†, Ernesto Pereda‡ and Santiago López§

**Unit of Clinical Neurophysiology, University Hospital La Candelaria, S/C de Tenerife, †Laboratory of Biophysics, Department of Physiology, Universidad de la Laguna, Tenerife, ‡Department of Basic Physics, Universidad de La Laguna, Tenerife and §Unit of Paediatrics, University Hospital La Candelaria, S/C de Tenerife, Spain*

Polysomnographic records from six healthy term neonates (age range: 8–14 weeks of life, mean age: 12 weeks) were studied to assess the existence of interdependence between the activity of the central nervous system (CNS) and the respiration during sleep. The monopolar EEGs corresponding to electrode positions Fp2, C4 and O2 (average as reference 0.25 Hz low-pass filter, 40 Hz high-pass filter) as well as the respiratory signal (RS) were recorded (128 Hz of sampling frequency for all the signals). From each baby, two simultaneously recorded stationary and artifact-free segments of each signal (8192 samples each) during waking (AW), active sleep (AS) and passive sleep (PS). A window of 512 samples was slid along the segments every ten samples and the total power of the low frequency (0.5–8 Hz) and the high frequency band (8.5–40 Hz) of each electrode as well as the total power of the RS peak were obtained for each window. The interdependence between the corresponding power signals of each electrode and that of the RS were then assessed by means of the Arnhold index S (Arnhold *et al.* 1999), after reconstructing the state space of each power signal by time delay embedding ($\mu = 10$, $\tau = 5$, $\kappa = 12$ for the calculation of S). The significance of the index and the nature of the interdependence were checked by the multivariate surrogate data test (Pereda *et al.* 2001).

The results showed that there existed significant interdependence between the power in the low frequency band (all the electrodes) and the power of the RS in all the situations. A MANOVA test was used to check the existence of differences among the different electrodes and situations, which were further analysed by Scheffé's *post-hoc* test. They were considered significant if $P < 0.05$.

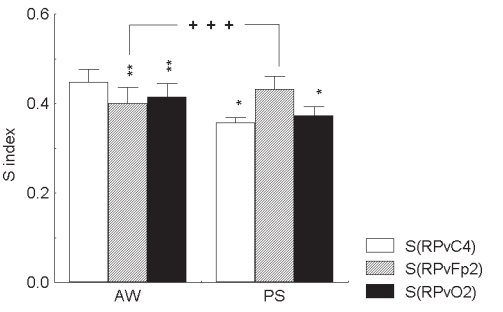

Figure 1. Mean values (± S.E.M.) of the S index between the respiratory power and the power in the low frequency band (electrodes Fp2, O2 and C4) during the waking state (AW) and passive sleep (PS). Asterisks indicate differences against C4 (AW) or Fp2 (PS) (*$P < 0.05$; **$P < 0.01$), while crosses indicate differences between both states (+++$P < 0.001$).

Figure 1 shows that the interdependence was greater for the central electrode during AW. However, during PS it was greater for the frontal electrode. No differences were found during AS. The *post hoc* test showed that this change was due to the

significant increase of the interdependence in the frontal electrode from AW to PS ($P < 0.001$), since the values of S for the other two electrodes did not change.

In adult humans, by using the correlation dimension of the EEG and that of respiratory movements, a decrease of the EEG complexity has been reported, which may be associated with an increased regularity of breathing in deep sleep (Burioka *et al.* 2001). Our results in neonates – obtained from multivariate analysis of both EEG and respiratory activities – are in accordance with these findings. In addition, we have demonstrated that in neonates during PS there exists a non-linear synchrony between the frontal EEG and the respiratory activity. This result may be important for the assessment of pathological situations in neonates, especially those dealing with respiratory alterations such as the sudden infant death syndrome.

Arnhold A *et al.* (1999). *Physica D* **134**, 419–430.

Burioka N *et al.* (2001). *Clin Neurophisiol* **112**, 1147–1153.

Pereda E *et al.* (2001). *Physica D* **148**, 147–158.

This work was supported by the FIS grant PI 020194 and the MCyT grant BFI2002-01159.

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

P87

Effects of the inhibition of Na^+/H^+ exchanger on ventricular fibrillation characteristics in normoxia conditions: an experimental study in isolated rabbit heart

L.M. Such, A. Blanco, F.J. Chorro*, L. Such, R. Salvador, J.M. Núñez, E. San Valero, R. Ferrandis and A. Alberola

*Departments of Physiology and *Medicine, Valencia University, 46010 Valencia, Spain*

It has been reported that Na^+/H^+ exchanger inhibition exerts beneficial effects on ischemic and reperfused myocardium, against arrhythmias (Gazmuri *et al.* 2001). In the present study, we analyse the effect of inhibition of this exchanger on ventricular fibrillation (VF) induced by pacing, in order to assess modifications of the intrinsic dominant frequency (FrD) of VF as a characteristic of this arrhythmia.

All the procedures were performed in accordance with the agreements of the European Convention of Strasbourg of March 18, 1986 (Instrument of ratification of 8/2/89, Official State Bulletin of 10/25/90). After anaesthetizing, heparinizing and killing 10 NZW rabbits (2.5 ± 0.4 kg), the heart was excised and immersed in a cold (4 °C) Tyrode solution; after, it was connected to a Langendorff system through the aorta (Tyrode pressure and temperature, 60 mmHg and 37 ± 0.5 °C, respectively). Ventricular stimulation electrode and atrial recording electrode were placed on ventricle and atrium, respectively. One plaque with 121 recording electrodes was placed on the left ventricle for ventricular electrograms. VF was induced by pacing at increasing frequencies. Two minutes after VF was induced, 5-(*N*-ethyl,*N*-isopropil) amiloride (EIPA), a well-known inhibitor of the Na^+/H^+ exchange system, was infused into the aortic root (0.04 μM) in the treated group. The analysis of dominant frequency was performed with data blocks of 2048 points (sampling rate = 1 kHz) before starting the main steps. FrD was obtained for each block. Data processing was performed with Matlab software on a Hewlett-Packard 712/80 platform. Two groups were used: a group of isolated hearts not treated with EIPA (control), and a group of isolated hearts treated with EIPA (treated). Student's unpaired t test was used to compare the results between both groups. Statistical significance was accepted when $P < 0.05$.

Mean values and standard deviation corresponding to mean FrD, maximum FrD, and minimum FrD values are shown in Table 1.

Table 1

	Mean FrD	Maximum FrD	Minimum FrD
Control	18.31 ± 3 (10)	21.75± 6 (7)	12.89 ± 2 (7)
Treated	15.93± 5 (10)	18.98± 5 (10)	11.99 ± 3 (10)

Values are given in hertz. Number of experiments is given in parentheses.

The inhibition of the Na^+/H^+ exchange system by EIPA did not modify FrD of ventricular fibrillation in normoxic conditions, as occurs with other intrinsic electrophysiological parameters, i.e. ventricular refractoriness, previously investigated.

Gazmuri RJ *et al.* (2001). *Circulation* **104**, 234–239.

This work was supported by grant no. PM98-0204 of Ministerio de Ciencia y Tecnología and by grant no. GV99-136-1-04 of Generalitat Valenciana.

All procedures accord with current National guidelines.

P88

Effect of chronic intermittent asphyxia on platelet function

M. Dunleavy*, M. Dooley†, D. Cox† and A. Bradford*

**Departments of Physiology and †Clinical Pharmacology, Royal College of Surgeons in Ireland, St Stephen's Green, Dublin 2, Ireland*

Obstructive sleep apnoea patients have increased platelet activity (Sanner *et al.* 2000). Acute (Li & Guo, 1996) and chronic continuous hypoxia (Nakanishi *et al.* 1997) are known to affect platelet function and number but the effects of chronic intermittent asphyxia (CIA), as occurs in obstructive sleep apnoea, are not known. In this study, we test the hypothesis that CIA causes changes in platelet function and number.

Wistar rats (CIA, $n = 13$) were exposed to alternating periods of normoxia and asphyxia twice a minute, 8 hours per day for 3 weeks. Controls (C, $n = 16$) were given air at the same flow rates as the CIA group. After 3 weeks, rats were anaesthetized (pentobarbitone, 60 mg kg^{-1} I.P.), artificially ventilated and thoracotomized. Blood was withdrawn from the left ventricle and analysed for platelet count, platelet activation (CD62p expression), response to low dose ADP (loss of single platelets) and closure time in the PFA 100 test, a test of blood coagulation. Animals were killed by anaesthetic overdose. All procedures were performed in accordance with national legislation under the Cruelty to Animals Act, 1876 and EU Directive 86/609/EC. Values are expressed as means ± S.D. and compared using ANOVA or the Mann-Whitney U test, ($P < 0.05$ taken as significant) where appropriate.

After the 3-week treatment, there was a significant difference in body weight between the two groups. There was no difference in platelet count between the two groups (C: 880.4 ± 80.3; CIA: $914.1 \pm 127.1 \times 10^3$ μl^{-1}) or in the reduction in count in response to ADP (reduced to 212.5 ± 193.5 *vs.* $190.7 \pm 129.3 \times 10^3$ μl^{-1}, C *vs.* CIA). There was no difference in the PFA closure time (C:

86.2 ± 27.4; CIA: 81.7 ± 20.1 s) or in the % of platelets positive for CD62 (C: 7.4 ± 9.2; CIA: 5.7 ± 3.1 %).

In conclusion, unlike chronic continuous hypoxia (Nakanishi *et al.* 1997), CIA did not cause a reduction in platelet number and had no effect on platelet activation. These results do not support a role for CIA in the changes in platelet function observed in obstructive sleep apnoea.

Li X & Guo X (1996). *Acta Physiog Sin* **48**, 457–463.
Nakanishi K *et al.* (1997). *J Pathol* **181**, 338–346.
Sanner BM *et al.* (2000). *Eur Respir J* **16**, 648–652.

This work was supported by the Royal College of Surgeons in Ireland.

All procedures accord with current National guidelines.

P89

Inhibition of rat spinally projecting PVN neurones by the neurosteroid tetrahydroxycorticosterone

R. Barrett-Jolley* and S. Pyner†

**Department of Physiology, University of Birmingham, UK and* †*School of Biology and Biomedical Science, University of Durham, UK*

The paraventricular nucleus of the hypothalamus (PVN) is central to integration of the stress response. It consists of neurones which release CRF, thus controlling cortisol secretion from the adrenal cortex, and neurones which project to sympathetic centres in the spinal cord and modulate cardiovascular function (Jansen *et al.* 1995). During acute stress, the characteristic increase in heart rate and blood pressure are accompanied by elevation in hypothalamic concentrations of neurosteroids such as tetrahydroxycorticosterone (THDOC, Paul & Purdy, 1992). Neurosteroids, in general, decrease sympathetic outflow, apparently by acting to enhance GABA inhibitory tone in the medulla (Laiprasert *et al.* 1998). However, THDOC has been reported to both enhance and inhibit GABA (Wetzel *et al.* 1999), and furthermore, the activity of neurosteroids on the $GABA_A$ channel is likely to be subunit dependent. We therefore chose to investigate whether THDOC modulates the activity of spinally projecting (pre-sympathetic) neurones (SPNs) of the PVN.

The methods used were similar to those described previously (Barrett-Jolley *et al.* 2000), but briefly: PVN SPNs were labelled by injection of a red (DiI) retrograde tracer into the spinal cord IML (T2-T4) of 3- to 4-week-old rats under general anaesthesia (I.P. injection of metatomidine–ketamine 0.3ml $(100\ g)^{-1}$, Home Office Procedure 40/2211.6) Approximately 1 week later, rats were humanely killed by an overdose of anaesthesia (I.P. sagittal) and 200 μm hypothalamic slices prepared. Results are given as means ± S.E.M.

Action currents were measured from identified SPNs, before and during the bath application of THDOC. Spontaneous action current frequency was reduced with a pIC_{50} of 6.9 ± 0.2 $n = 5$.

Under these recording conditions, THDOC could be acting directly on $GABA_A$ receptors of the recorded neurone, or indirectly via $GABA_A$ receptors of neurones projecting to the recorded neurone. To investigate whether SPNs actually express $GABA_A$ receptors, we combined an immunohistochemical study with retrograde labelling. We found all retrogradely labelled SPNs to express the predominant PVN $GABA_A$ α-subunit, α_2.

In whole-cell patch-clamp experiments, we found SPN $GABA_A$ currents (elicited by pressure injection of 300 μM GABA) to be enhanced by 1 μM THDOC to 148 ± 15 % $n = 5$ ($P < 0.05$, one-sample t test).

These results suggest that during stress, when concentrations of hypothalamic neurosteroids rise, pre-sympathetic neurones projecting from the PVN to the spinal cord will be inhibited by THDOC.

Barrett-Jolley R *et al.* (2000). *J Neurosci Met* **102**, 25–33.
Jansen AS *et al.* (1995). *Science* **270**, 644–646.
Laiprasert JD (1998). *Am J Physiol* **274**, 1–11.
Paul SM & Purdy RH (1992). *FASEB J* **6**, 2311–2322.
Wetzel CH *et al.* (1999). *Br J Pharmacol* **127**, 863–868.

This work was funded by the BHF.

All procedures accord with current UK legislation.

P90

High noradrenaline spillover in healthy humans acclimatised to high altitude

José A.L. Calbet

Departamento de Educación Física, Universidad de Las Palmas de Gran Canaria and Copenhagen Muscle Research Centre, Campus Universitario de Tafira, 35017 Las Palmas de Gran Canaria, Canary Islands, Spain

Chronic hypoxia is associated with elevated sympathetic activity and hypertension in patients with chronic pulmonary obstructive disease (Heindl *et al.* 2001; Imadojemu *et al.* 2002). However, the effect of chronic hypoxia on systemic and regional sympathetic activity in healthy humans remains unknown. To determine if chronic hypoxia in healthy humans is associated with hyperactivity of the sympathetic nervous system, we measured intra-arterial blood pressure (20-gauge femoral artery catheter), arterial blood gases, systemic and muscular noradrenaline spillover and vascular conductances in nine Danish lowlanders (4 females and 5 males) at sealevel and after 9 weeks of exposure at 5260 m (barometric pressure = 408 mmHg). Their mean (± S.E.M.) age, height and weight were 24.3 ± 0.5 years, 176 ± 3 cm and 74 ± 4 kg, respectively. The subjects were informed about the procedures and risks of the study before giving written informed consent to participate as approved by the Copenhagen-Fredriksberg Ethical Committee. Experiments were performed according to the Declaration of Helsinki. Differences in the measured variables among conditions were assessed with ANOVA for repeated measures, with the sex as a between-subjects factor with two levels. Student's paired t test was applied when appropriate to determine if the observed differences between the means were significant or not. Significance was accepted at $P < 0.05$.

Mean blood pressure was 28 % higher at altitude ($P < 0.01$) due to increases in both systolic (18 % higher, $P < 0.05$) and diastolic (41 % higher, $P < 0.001$) blood pressures. Cardiac output (indocyanin green injection) and leg blood flow (thermodilution, femoral vein catheter) were not altered by chronic hypoxia, but systemic vascular conductance was reduced by 30 % ($P < 0.05$). Plasma arterial noradrenaline (NA) and adrenaline concentration were 3.7- and 2.4-fold higher at altitude, respectively ($P < 0.05$). The elevation of plasma arterial NA concentration was caused by a 3.8-fold higher whole-body noradrenaline release ($P < 0.001$) since whole-body noradrenaline clearance was similar in both conditions. Leg NE spillover was increased similarly (×3.2, $P < 0.05$). These changes occurred despite systemic O_2 delivery

being greater after altitude acclimatisation than at sea level, due to 37% higher blood haemoglobin concentration.

In summary, this study shows that chronic hypoxia causes increased systemic arterial pressure and massive activation of the sympathetic nervous system in healthy humans, despite improved oxygenation with acclimatisation. Strikingly, sympathetic activation reaches levels similar to those observed in chronic heart failure patients (Kaye *et al.* 1995). It is particularly important to establish the functional and pathophysiological consequences that may be derived from the chronic hypoxia induced sympathetic hyperactivity.

Heindl S *et al.* (2001). *Am J Respir Crit Care Med* **164**, 597–601.

Imadojemu VA *et al.* (2002). *Am J Respir Crit Care Med* **165**, 61–66.

Kaye DM *et al.* (1995). *J Am Coll Cardiol* **26**, 1257–1263.

Special thanks are given to Harriet Wagner, Carsten Nielsen, Karin Hansen and Birgitte Jessen for their excellent technical assistance. This study was supported by a grant from The Danish National Research Foundation (504-14).

All procedures accord with national and local guidelines and the Declaration of Helsinki.