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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C12 and PC61

Area-specific reorganization of catecholamine release from C1 and A2 neurones of spontaneously hypertensive rats

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Central catecholaminergic (CAergic) neurones, in particular areas C1 and A2, are associated with blood pressure regulation. Links between their activity and central sympathetic outflow have been documented. Since sympathetic drive is elevated in essential hypertension, both in human patients and spontaneously hypertensive rats (SHR), hyperactive central CAergic transmission may be an underlying causative factor.

We compared transmitter release characteristics and electrophysiological properties of C1 and A2 neurones of normotensive Wistar rats (WR) with SHR *in vitro*. Organotypic brainstem slice cultures were transduced with adenoviral vectors to express EGFP specifically in CAergic neurones [1]. CAergic neurones were imaged using a Leica SP confocal microscope and recorded in whole-cell configuration with patch electrodes containing the Ca^{2+} indicator Rhod-2 [2]. Electrophysiological characteristics of neurones and their intracellular $[Ca^{2+}]$ ($[Ca^{2+}]i$) responses to angiotensin II (AngII; 200 nM) were determined. For analysis of quantal catecholamine (CA) release characteristics, microamperometry was carried out in separate experiments where carbon fiber microelectrodes were placed onto single varicosities. Oxidation currents were evaluated as in [3]. Microamperometric analysis at C1 release sites in WR and SHR (n=6 each) revealed a striking increase of the relative contribution of large (>0.5 pC) release events in SHR (from 57% to 86% of total CA release) as compared to WR, while contribution of small events (median ~0.025 pC) was diminished (from 43% to 14% of total). This represents a significant redistribution of the mode of release in favour of large quanta in C1 ($p<0.05$ Chi-square for all fractions). Interestingly, in WR, 48% of C1 neurones (n=44) were spontaneously active while in SHR the majority (90%) were silent (n=10). AngII raised $[Ca^{2+}]i$ in silent C1 neurones of WR and SHR (in 61% and 55%, respectively). This increase was not significantly different between WR and SHR cells (+24 ± 1.8% and +34 ± 8%; p=0.26; Student's t-test). For area A2, the distribution of various types of release events was similar between WR and SHR. Here, comparable subpopulations of active neurones were found in WR (38%; n=8) and SHR (22%; n=9). For active as well as silent cell groups, the effect of AngII on $[Ca^{2+}]i$ was markedly reduced in SHR (+5 ± 3%) compared to WR (+29 ± 9%; $p<0.05$, Student's t-test).

In conclusion, in SHR we have uncovered a significant shift in release characteristics of C1 varicosities such that the bulk of CA is released in massive packages in excess of 10^6 molecules. When released in large packets, transmitter may spread further through extracellular space, opening up a wider range of signalling targets for C1 neurones in SHR.

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C13 and PC62

Altered expression of neuropeptide Y Y_1, Y_2 and Y_5 receptors and their functional consequences in the tail artery of diabetic rats

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Vascular dysfunction is a common consequence of diabetes mellitus. Alterations in sensitivity and/or responsiveness to the 'sympathetic triad' of neurotransmitters (noradrenaline, ATP and NPY) may underlie functional abnormalities of diabetic blood vessels (Chow *et al*., 2001; Tefamariam, 1995). Recently, we have found a greater contribution to vasoconstriction from NPY Y_1 and Y_2 receptor subtypes in arteries from diabetic rats (Kerlin *et al*., 2006). In this study we have further investigated alterations in expression of NPY receptor subtypes and their associated functional responses in diabetic rat tail arteries.

Diabetes was induced in Sprague-Dawley rats (8 weeks old) by injection of streptozotocin (i.p., 60 $mg.kg^{-1}$). Tail artery was excised from humanely dispatched rats at 20 weeks. Receptor expression was determined at both mRNA and protein level using RT-PCR (normalized to GAPDH mRNA) and Western blotting (standardized to β-actin) respectively. Sections of proximal tail artery (3–5 mm, endothelium-denuded) were suspended in tissue baths perfused with Krebs-Hansleit solution for isometric contractile studies.

NPY Y_1 and NPY Y_2 mRNA receptor expression was significantly increased, 2.84 ± 0.47 fold (n=5, $P<0.05$; unpaired Student's *t*-test) and 2.78 ± 0.55 fold (n=5, $P<0.01$), respectively, in diabetic rat tail artery relative to the age-matched control. Parallel increases in NPY Y_1 (n=5, $P<0.05$, 5.76 fold) and NPY Y_2 (n=5, $P<0.01$, 1.73 fold) receptor expression were observed at protein level. A 5.45 ± 0.21 fold decrease in NPY Y_5 receptor mRNA expression (n=5, $P<0.01$) was detected in diabetic rat tail artery relative to the control. No change was observed in sympathetically-evoked constrictions (20 impulses at 20 Hz, pulse duration 1 ms, supra-maximal voltage) from control and diabetic tail artery upon the application (5 nM) of the NPY Y_5 antagonist CGP71683.

We have now confirmed at protein level, in addition to mRNA level, an increase in expression of NPY Y_1, and Y_2 receptors in diabetic rat tail arteries, which may explain their increased contribution to vasoconstriction seen previously (Kerlin *et al*., 2006). Our on-going studies have shown that NPY Y_5 receptors are not involved in vasoconstriction in control or diabetic tissues, although NPY Y_5 mRNA is down-regulated in diabetes. Therefore, of these changes, only the increase in NPY Y_1 and NPY Y_2 expression are of consequence for contraction.

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C14 and PC63

A repressor of neuronal genes inhibits cardiac hypertrophy

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The most common disorder causing sudden cardiac death in young people is the cardiac disease, hypertrophic cardiomyopathy (HCM). Recent studies have suggested that HCM is more common than previously reported and it is now estimated that approximately l in every 500 people in the UK suffer from the disease. HCM is characterised by cardiac hypertrophy, an abnormal thickening of the heart muscle and while most affected individuals show few or no symptoms, others suffer heart failure, arrhythmias, and sudden death. The reason for the emergence of these symptoms in some people, but not others, remains unknown but since the transcriptional levels of a number of genes are indicative of the disease phenotype, the control of transcriptional programs could provide one mechanism to alleviate HCM.

The genes encoding the brain and atrial natriuretic peptides (BNP and ANP) are normally highly expressed in the foetal heart with levels reducing during development. High levels of expression of these genes are observed in adult ventricular myocytes in cardiac hypertrophy. One transcription factor that is important in repressing ANP and BNP expression in the normal adult heart is the Repressor Element 1-Silencing Transcription factor (REST). The aim of this study was to investigate the molecular mechanisms of REST-mediated repression and its potential role in hypertrophy. This was achieved by interrogating protein-DNA interactions and chromatin modifications at REST binding sites by chromatin immunoprecipitation and RT-PCR. REST represses its target genes by recruiting two distinct corepressor complexes that include histone deacetylases (HDAC1, HDAC2) and a H3 lysine 4-specific demethylase (LSD1). Inhibition of REST function resulted in an increase in ANP and BNP gene expression that correlated with increases in histone acetylation and dimethylation of H3 lysine 4 at the ANP and BNP promoters. Additionally, increasing REST expression in adult rat cardiomyocytes prevented increases in ANP and BNP expression by the hypertrophic agonist, endothelin-1. This data provides evidence that a therapeutic strategy aimed at augmenting REST and/or the action of its corepressors may be effective in treating cardiac hypertrophy.

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C15 and PC64

Two mechanisms mediate the noradrenergic slow depolarization in rat tail artery

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In rat tail artery, electrical stimulation of the sympathetic nerves evokes both an ATP-mediated excitatory junction potential (EJP) and a slower noradrenaline-mediated depolarization (NAD). Here we investigated the mechanisms underlying the NAD. Segments of proximal tail artery isolated from rats were mounted in a 1 ml recording chamber and the perivascular axons were electrically stimulated via a suction electrode applied to the proximal end. Intracellular recordings were made from the vascular smooth muscle cells. Application of the α_1-adrenceptor antagonist prazosin (0.1 μM, n = 6) slowed the rising phase of the NAD but did not change its amplitude or duration. In contrast, the α_2-adrenoceptor antagonist idazoxan (1 μM, n = 6) did not change the onset of the NAD but it did reduce its amplitude and duration. The combined application of prazosin and idazoxan abolished the NAD. In the presence of prazosin, the NAD was completely blocked by the K_{ATP} channel blockers, glybenclamide (10 μM, n = 6) and PNU 37883A (5 μM, n = 6). These agents also produced membrane depolarization. The NAD remaining when α_2-adrenoceptors were blocked was not affected by glybenclamide (10 μM, n = 5). In rat tail artery, the time constant of decay of the EJP is determined by the membrane time constant (Cassell *et al.*, 1988). The time constant of decay of EJPs evoked at the peak of the idazoxan-resistant NAD was prolonged (relative change 1.16 ± 0.03, $P < 0.01$, n = 6) suggesting that the α_1-component of the depolarization is also mediated by closure of K^+ channels. However, this component was not inhibited by broad-spectrum K^+ channel blockers (tetraethylammonium, 4-aminopyridine, Ba^{2+}). The idazoxan-resistant NAD was also unaffected by the Cl^- channel blockers, 9-anthracene carboxylic acid (100 μM, n = 4) and niflumic acid (10 μM, n = 3). These findings indicate that the NAD has two components; one which is due to activation of α_1-adrenoceptors and the other to activation of α_2-adrenoceptors. The α_2-adrenoceptor-mediated component is due to closure of K_{ATP} channels whereas α_1-adrenoceptor mediated component is most likely mediated by closure of another type of K^+ channel.

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