Research Paper

The transcriptome of the medullary area postrema: the thirsty rat, the hungry rat and the hypertensive rat

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The area postrema (AP) is a sensory circumventricular organ characterized by extensive fenestrated vasculature and neurons which are capable of detecting circulating signals of osmotic, cardiovascular, immune and metabolic status. The AP can communicate these messages via efferent projections to brainstem and hypothalamic structures that are able to orchestrate an appropriate response. We have used microarrays to profile the transcriptome of the AP in the Sprague–Dawley (SD) and Wistar–Kyoto rat and present here a comprehensive catalogue of gene expression, focusing specifically on the population of ion channels, receptors and G protein-coupled receptors expressed in this sensory tissue; of the G protein-coupled receptors expressed in the rat AP, we identified ∼36% that are orphans, having no established ligand. We have also looked at the ways in which the AP transcriptome responds to the physiological stressors of 72 h dehydration (DSD) and 48 h fasting (FSD) and have performed microarrays in these conditions. Comparison between the DSD and SD or between FSD and SD revealed only a modest number of AP genes that are regulated by these homeostatic challenges. The expression levels of a much larger number of genes are altered in the spontaneously hypertensive rat AP compared with the normotensive Wistar–Kyoto control rat, however. Finally, analysis of these 'hypertension-related' elements revealed genes that are involved in the regulation of both blood pressure and immune function and as such are excellent targets for further study.

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The area postrema (AP) is situated on the mid-line dorsal surface of the medulla at the level of the obex and is a part of the dorsal vagal complex that also includes the nucleus tractus solitarii (NTS) and the dorsal motor nucleus of the vagus (DMV; Borison, 1984, 1989). It is one of the sensory circumventricular organs (CVOs) and as such is distinct from the rest of the CNS in its extensive vascular supply, which is derived from specialized capillaries lacking the normal blood–brain barrier (Gross, 1991). The AP has also been shown to contain receptors for, and/or respond to, a variety of different peripheral signals, including regulatory peptides (e.g. adiponectin, adrenomedullin, angiotensin, amylin, cholecystokinin, ghrelin, glucagon-like peptide 1, endothelin and vasopressin; Goke *et al.* 1995; Allen & Ferguson, 1996; Sun & Ferguson, 1997; Riediger *et al.* 2002; Barth *et al.* 2004; Fry *et al.* 2006; Fry & Ferguson, 2009), steroids (e.g. oestradiol; Li & Hay, 2000; Pamidimukkala & Hay, 2003) and ionic constituents of the extracellular environment (e.g. osmolarity, Ca^{2+} and Na^{+}; Ferry *et al.* 2000; Ho *et al.* 2007).

Despite the early view of the AP as primarily the chemoreceptor trigger zone responsible for the control of emesis, accumulating data now suggest that this CVO plays important roles in sensing circulating signals involved in the integrative regulation of multiple components of the autonomic nervous system. Functional roles for the AP in sensing immune (Laflamme & Rivest, 2001), metabolic (Edwards & Ritter, 1981; Contreras *et al.* 1982;

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Bird *et al.* 1983) and cardiovascular signals (Barnes & Ferrario, 1981; Ferguson & Marcus, 1988; Bhatnagar *et al.* 1999) have all been identified. To investigate this, we used whole-genome microarray analysis to provide a comprehensive, unbiased identification of functionally discrete groups of transcripts in the AP from control, 72 h dehydrated and 48 h fasted Sprague–Dawley (SD) rats and from spontaneously hypertensive rats (SHRs) and Wistar–Kyoto (WKY) rats.

Methods

Animals

All experimental procedures were approved by the University of Bristol Ethical Review Committee and were carried out under UK Government licence in accord with the Animals (Scientific Procedures) Act 1986. Adult male SD rats, WKY rats and SHRs (10–12 weeks old; Harlan Sera-lab, Loughborough, UK) were maintained in standardized temperature ($22 \pm 1^{\circ}$C), humidity ($50 \pm 5\%$) and diurnal conditions (10 h light and 14 h dark; lights on at 07.00 h). The control group of animals had access to both food and drinking water for the duration of the experiment. Three (dehydration group) or 2 days (food deprivation group) before tissue extraction, water bottles or food, respectively, were removed at 11.00 h from separate groups of animals. Following 72 h of water deprivation or 48 h of total food deprivation, the rats were killed (between the hours of 10.00 and 13.00 h). Control animals were also killed at the same time (between 11.00 and 13.00 h) each day. Each single microarray represents five animals.

Tissue collection and array processing

Rats were stunned and then decapitated with a small animal guillotine (Harvard Apparatus, Holliston, MA, USA). The brain was rapidly removed from the cranium and placed in ice-cold artificial cerebrospinal fluid containing (mM): 124 NaCl, 2 KCl, 1.25 KH_2PO_4, 2.0 $CaCl_2$, 1.3 $MgSO_4$, 20 $NaHCO_3$ and 10 glucose. Brainstem dissections were carefully trimmed, glued on a mounting block and supported with solidified agar. Mounted brainstems were then submerged into the sectioning bath of a vibratome (Vibratome Bannockburn, IL, USA), and coronal sections 300–400 μm thick were prepared, placed in Hibernate medium (Brain Bits, Springfield, IL, USA) containing 1× B27 supplement (Invitrogen, Burlington, ON, USA). The AP was then carefully hand-microdissected away from surrounding tissue under a dissecting microscope, by an experienced anatomist using a brain map for reference (Paxinos & Watson, 2005; see also 'Key & Anatomy' in supplemental data available online at http://www.vasopressin.org/#/data-bank/3755442). Consistency between samples was maintained through the use of a single anatomist for all AP dissections in this experiment. After isolation, the samples were immediately immersed in RNAlater (Ambion, Huntingdon, UK). Tissue processing, RNA extraction, amplification, hybridization and washing were carried out according to Hindmarch *et al.* (2008), and transcriptomic services were provided by Source-Bioscience (Nottingham, UK).

Data analysis

The raw data (.CEL files) from each of the 23 rat Genechip 230 2.0 microarrays was then loaded into GeneSpring® GX11 (Agilent Technologies, Stockport, UK), where they were summarized with Mas5 (which incorporates a scaling normalization) and transformed to the median of all samples. All raw data have been submitted to the NBCI Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo, Accession number: GSE26660) and supplemental files S1-S16 are available here: http://www.vasopressin.org/#/data-bank/3755442. Data were used to compile catalogues of genes flagged as 'Present' in each independent microarray set from each of the five experimental groups; control SD (SD; S2, $n = 4$), dehydrated (DSD; S3, $n = 4$), fasted (FSD; S4, $n = 5$), normotensive (WKY; S5, $n = 5$) and hypertensive (SHR; S6, $n = 5$). Any data points called 'Marginal' or 'Absent' in any microarray were removed at this stage. To provide a basis for further analysis, the individual lists of genes considered Present in each condition were combined in a manner that excludes repetition of any single probe, either SD + WKY or SD + FSD + DSD + WKY + SHR. Note that while by definition this list represents genes that are flagged as Present in one or more of the experiments, some genes may be considered Marginal or Absent in one or more of the other conditions. Statistical analysis comprised Welch ANOVA with Tukey's *post hoc* test and Benjamini–Hochberg (B&H) multiple testing correction (P value to 0.05). Appropriate comparisons that resulted from the *post hoc* test were subsequently filtered with a 1.5-fold cut-off. Gene ontology (GO) analysis was performed on the WKY *versus* SHR comparison ($P < 0.05$ B&H, >1.5-fold) and used Benjamini–Yekutieli multiple test correction $P < 0.05$.

Results

Assessment of the variability of transcriptome data

In this study, we have used outbred Sprague–Dawley rats and made comparsions with inbred SHRs and WKY rats. To minimize the consequences of any increase in genetic variability that might be found in the outbred population, we have, for each condition, pooled five animals per chip

and used four or more independent microarray replicates. We have measured variability in our microarrays by comparing average correlation coefficients using all data from each chip. Across all 23 microarrays, the correlation is exceptionally strong ($r = 0.96$; $\sigma_M = 0.0005$), a trend repeated between individual comparisons (S1).

Catalogues of gene expression in the AP

For each condition, we then established lists of genes that are flagged as Present in all of the microarrays for that condition, resulting in 15,396 genes in the SD (S2), 16,088 genes in the DSD (S3), 15,616 genes in the FSD (S4), 15,739 genes in the WKY (S5) and 15,563 genes in the SHR (S6; Fig. 1).

Gene expression conservation between rodent species

We hypothesize conservation of AP gene expression between two closely related species, such as the rat and the mouse. We have examined this possibility, while at the same time providing additional validation of our array findings by comparing mouse (C57BL/6J) AP-enriched genes (http://www.brain-map.org/, Lein *et al.* 2007; Glattfelder *et al.* 2008) with our transcriptome catalogues in the SD (S2) and WKY rat (S3). We find excellent correlation between rat and mouse AP gene expression; of the 50 genes whose expression has been mapped in the mouse AP, 42 are represented on the rat chip, of which 37 (88%) are flagged as Present in at least one of our experimental groups (the majority in all five groups; S10).

Area postrema transcriptome comparison between rat strains

In order to investigate strain differences between the inbred WKY rat and the outbred SD rat, we compiled a new list that combined the SD (S2) and WKY (S3) Present lists. We filtered this new list for those genes that are significantly different between these two strains (Welch t-test B&H, $P < 0.05$) and then applied an enrichment cut-off of 1.5-fold to reveal 460 genes (S11) whose expression is enriched in the SD AP compared with the WKY, and 875 genes (S12) enriched in the WKY AP compared with the SD. The majority of the genes in the AP are, however, well conserved between the two strains; 13,911 genes (data not shown) are considered to be Present in both the SD and the WKY AP.

Comprehensive catalogues of AP gene expression. The sensory function of the AP led us to focus on specific broad groups of genes whose expression within the SD or WKY AP directly contributes to this function, namely receptors (including G protein-coupled receptors; GPCRs) and ion channels. Lists of genes that are represented on the Affymetrix array were isolated using the wildcard operator terms 'receptor' (1409), 'GPCR', 'GPR' and 'G protein-coupled receptor' (324) or 'channel' (300). When we compared the lists of putative receptors with the SD + WKY list, we identified a population of

CTSD	DHSD	FSD	WKY	SHR	CTSD+DHSD+FSD+WKY +SHR
15,396 (S1)	16,088 (S2)	15,616 (S3)	15,739 (S4)	15,642 (S5)	17,536

Comparison	Regulated >1.5-fold (P<0.05)
CTSD v. DHSD	53 (S12)
CTSD v. FSD	29 (S12)
WKY v. SHR	315 (S13)

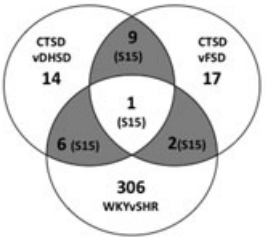


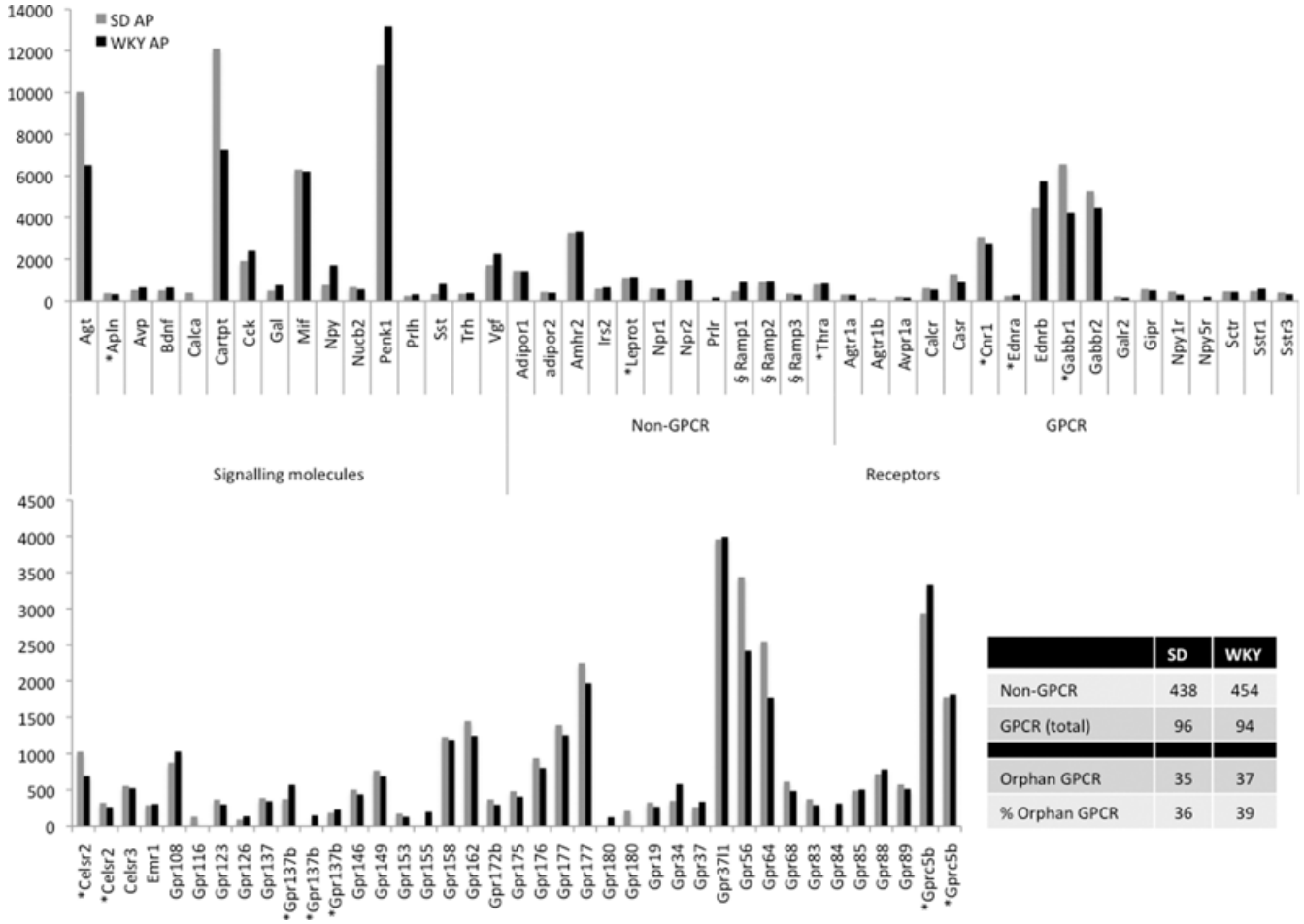
Figure 1. The transcriptome of the medullary area postrema
The transcriptome of the area postrema (AP) was interrogated using Affymetrix 230 2.0 Rat Genechip microarrays with 31,099 individual probe sets. Data from the AP of control (CTSD), 72 h dehydrated (DSD) and 48 h fasted Sprague–Dawley rats (FSD), Wistar–Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) was loaded into Genespring 11 and summarized into a single experiment. Lists of genes flagged as 'Present' were established for each condition and combined to form an experimental list of 17,562 genes from which statistical and fold-change analysis could be performed (Welch ANOVA, $P < 0.05$, fold change >1.5). The Venn diagram shows the comparison of those genes significantly regulated by greater than 1.5-fold by 72 h dehydration or 48 h fasting in the SD rat AP or those significantly regulated by greater than 1.5-fold in the SHR AP compared with the WKY rat AP.

579 receptors in the AP of either the SD or the WKY (or both), of which 107 were identified as GPCRs (S7; Fig. 2). Comparison of the channel list revealed 108 genes annotated as 'channel' in their gene title or symbol (S8; Fig. 3). We have also selected signalling molecules that may act as ligands for these receptors and whose expression in the AP has previously been demonstrated (Fig. 2).

Steady-state changes in transcriptome expression. We applied statistical testing (Welch ANOVA + Tukey *post hoc* test and B&H, $P > 0.05$) to our SD + FSD + DSD + WKY + SHR Present list. Following a 1.5-fold cut-off, 53 genes in the DSD AP (S13) and 29 genes in the FSD AP (S13) were regulated compared with the SD AP (Fig. 1). In the SHR AP (S14), 315 genes were regulated compared with the WKY AP. Filtering of this list revealed four genes that converge on known rat quantitative trait loci (QTL; Fig. 4), and GO analysis ($P < 0.05$) revealed 10 GO terms that are enriched in this list of genes (Fig. 5). Venn analysis (Fig. 1) was used to compare gene regulation between these three conditions, revealing only one gene common to all three conditions, nine in common between fasting and dehydration, six in common between dehydration and hypertension and only two in common between hypertension and fasting.

Discussion

We have used microarrays to profile the transcriptome of the rat AP from the SD rat, the DSD rat, the FSD rat, the normotensive WKY rat and the SHR. We have confirmed that the experimental variability is very low, that the majority of genes are commonly expressed between the strains studied here and that that gene expression is



	SD	WKY
Non-GPCR	438	454
GPCR (total)	96	94
Orphan GPCR	35	37
% Orphan GPCR	36	39

Figure 2. Receptor and signalling-molecule expression in the area postrema
The top graph shows a selection of known and novel signalling molecules, G protein-coupled receptors (GPCRs) and non-GPCRs, together with their relative expression levels in the AP of the SD and WKY rats. * Multiple probe sets for gene; § accessory proteins that confer receptor specificity. The bottom graph shows 39 orphan GPCRs (defined by IupHR; http://www.iuphar-db.org/DATABASE/ReceptorFamiliesForward?type=GPCR), together with their relative expression levels in the SD and WKY AP. Table (bottom right) shows the number of non-GPCRs, the number of GPCRs, and the percentage of orphan GPCRs in the SD and WKY AP.

conserved between the rat and the mouse AP. We present our findings here in the light of the literature, which we have used to partly validate our catalogues. Conformation of known AP-expressed genes instills confidence regarding the robustness and completeness of our transcriptomic analysis. Such is our confidence that we have made our data fully available to the scientific community via the Gene Expression Omnibus (GSE26660) so that they can be independently scrutinized and validated.

The literature confirms that several of the identified receptors are expressed in the AP, such as the angiotensin II type 1a receptor (*Agtr1a*; Lenkei *et al.* 1998; Huang *et al.* 2003), vasopressin 1a receptor (*V1aR*; Tribollet *et al.* 1999; Yang *et al.* 2006), atrial natriuretic peptide receptor a and b (*Npr1/2*; Konrad *et al.* 1992*a*,*b*), prolactin receptor (*Prlr*; Mangurian *et al.* 1999), neuropeptide Y1 (*Nyp1r*) and Y5 (*Nyp5r*) receptors (Dumont *et al.* 1996; Dumont *et al.* 1998*a*,*b*), glucagon-like peptide-1 receptor (*Glpr*; Price *et al.* 2008), cannabinoid 1 receptor (*Cnr1*; Partosoedarso *et al.* 2003), adiponectin receptor (*AdipoR1/R2*; Fry *et al.* 2006) and receptor activity modifying protein (RAMP) mRNAs 1, 2 and 3 (Ueda *et al.* 2001). While these RAMPs are not strictly receptors, they are accessory proteins that confer receptor specificity; for example, all three RAMPs heterodimerize with the calcitonin receptor to form three different receptors for amylin, a hormone co-secreted with insulin (Christopoulos *et al.* 1999; Muff *et al.* 1999). Figure 2 also shows several novel receptors not previously described in the literature, which present interesting targets for future work, such as the γ-aminobutyric acid b (*Gabab*), secretin (*Sstr 1* and *3*), and somatostatin (*Sst*) receptors.

We also specifically present a list of GPCRs expressed in the AP. The GPCRs are the largest family of transmembrane proteins, with almost 2000 members in the rodent genome (Gloriam *et al.* 2007), the majority of which are odorant receptors. The GPCRs specifically bind a large variety of ligands, including neurotransmitters,

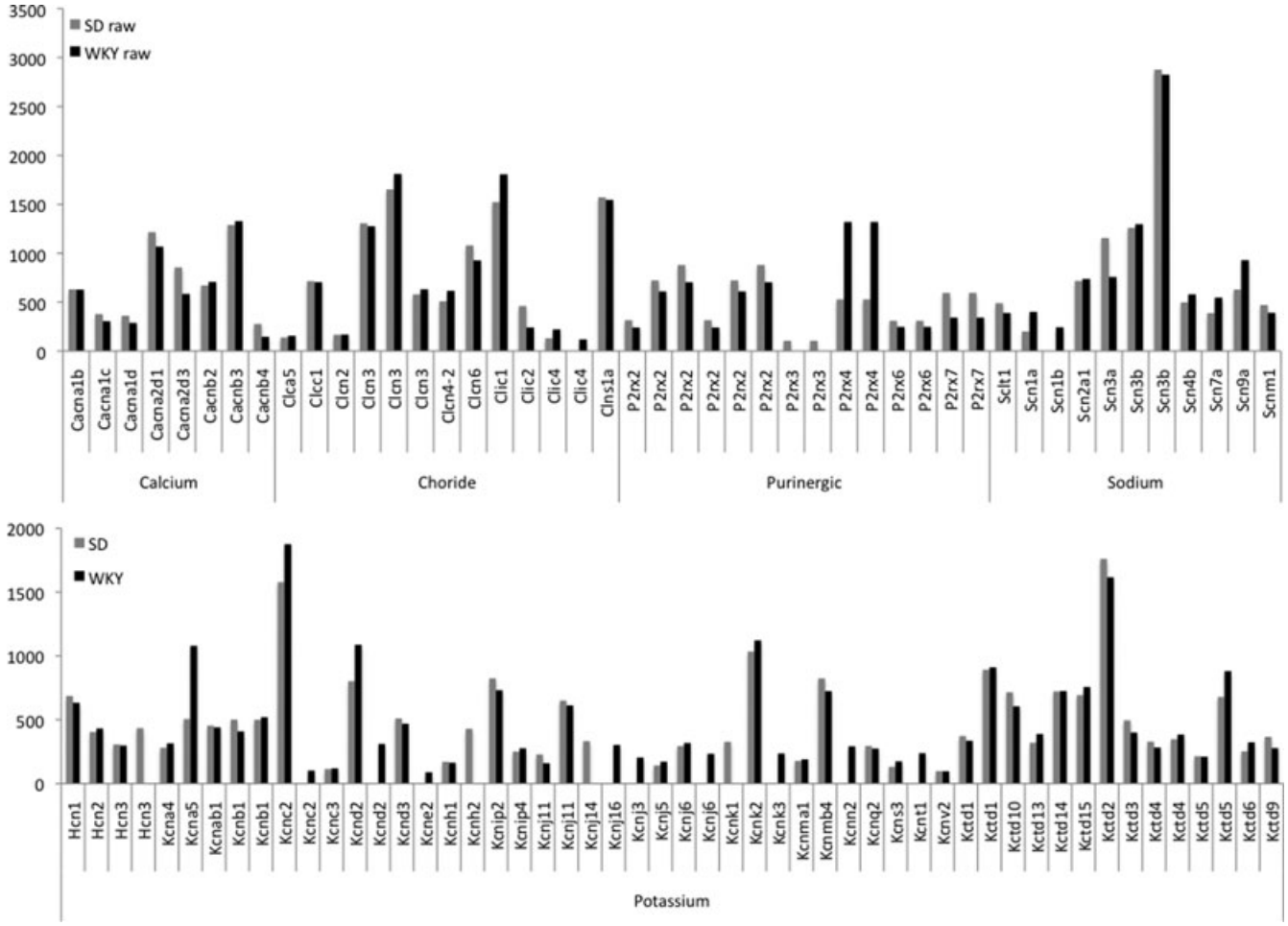


Figure 3. Channel expression in the area postrema
The top graph shows the broad groups of calcium, chloride, purinergic and sodium channels expressed in the area postrema (AP) of SD or WKY rats. The bottom graph shows potassium channel expression in the AP of the SD and the WKY AP.

Gene	loci	QTL symbol//QTL name//QTL trait//QTL marker (peak or flank)
Phosphorylase, glycogen, muscle	**Chr1q43**	Bp288 // Blood pressure QTL 288 // Blood pressure // peak
		Glom13 // Glomerulus QTL 13 // Renal pathology // peak
		Thym1 // Thymus enlargement QTL 1 // Gland mass // peak
		Thym4 // Thymus enlargement QTL 4 // Gland mass // flank
Acetyl-Coenzyme A acyltransferase 1	**Chr8q32**	Bp331 // Blood pressure QTL 331 // Blood pressure // peak
Tachykinin 1	**Chr4q21**	Eae11 // Experimental allergic encephalomyelitis QTL 11 // Brain/spinal cord inflammation // flank
		Hrtrt13 // Heart rate QTL 13 // Heart rate // peak
		Srn5 // Serum renin concentration QTL 5 // Renin concentration // flank
ATPase, Na+/K+ transporting, alpha 2 polypeptide	**Chr13q24-q26**	Tgl9 // Triglyceride level QTL 9 // Lipid level // flank
		Thym2 // Thymus enlargement QTL 2 // Gland mass // flank

Figure 4. Regulated genes in the spontaneously hypertensive rat AP that also converge on known rat quantitative trait loci (QTL)

GO Accession	Go Term	Corrected *p*-value	# Genes	GO Domains
GO:0050896|GO:0051869	response to stimulus	0.0028474003	50	Biological process
GO:0048002	antigen processing and presentation of peptide antigen	1.3626046E-8	9	Biological process
GO:0044459	plasma membrane part	0.047260944	28	Cellular component
GO:0042612	MHC class I protein complex	1.30432705E-8	8	Cellular component
GO:0042611	MHC protein complex	9.563295E-9	9	Cellular component
GO:0019882|GO:0030333	antigen processing and presentation	6.052448E-7	9	Biological process
GO:0008217	regulation of blood pressure	0.047260944	10	Biological process
GO:0006955	immune response	0.012578057	12	Biological process
GO:0005886|GO:0005904	plasma membrane	0.04747953	45	Cellular component
GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	9.563295E-9	8	Biological process

Figure 5. Gene ontology (GO) analysis results indicating the GO accessions that pass the corrected (Benjamini–Yekutieli multiple test correction) *P* value threshold to $P < 0.05$ (performed in GX11)
The GO accession and GO term correspond to the controlled vocabulary of gene function laid out by 'the Gene Ontology' (http://www.geneontology.org/). Within the list of genes differentially regulated by greater than 1.5-fold in the SHR AP compared with the WKY AP, 10 overlapping GO terms were enriched, including several GO terms corresponding to immune function and one corresponding to the 'regulation of blood pressure'.

peptide and non-peptide hormones, amino acids, ions, chemokines, lipids, peptides (including proteases) and photons, and as a result it is estimated that over 50% of all therapeutic agents target GPCRs (Dorsam & Gutkind, 2007). For the SD and WKY AP, we present catalogues of 96 and 94 GPCRs, respectively (S7; Fig. 2). Multiple probe sets for single GPCRs within this list may not represent 'unique' receptors, however; for example, there are three splice variants of the *GababR1* detected in the AP: *1a*, *1j* and *1f*. The $GABA_B$ R1 receptor isoforms may confer functional differences to the $GABA_B$ R1/R2 receptor heterodimer (Tiao *et al.* 2008). Interestingly, in the SD and the WKY AP, 35 and 37% (respectively) of the GPCRs are orphan GPCRs (as defined by the IupHAR GPCR database; IUPHAR, 2010), for which ligands have yet to be identified. Given the anatomical location of the AP and the lack of a normal blood–brain barrier, this list may contain important pharmacological targets and may elucidate potential novel functional roles for the AP.

Finally, we have established lists of channels whose mRNA is expressed within the AP of the SD and WKY rats. We confirm the previously reported presence of broad classes of ion channels (calcium, chloride, purinergic, sodium and potassium; S8; Fig. 3). Although some of these ion channel isoformsn, such as HCN, have previously been identified in AP (Milligan *et al.* 2006), this is the first comprehensive description of the ion channels expressed in AP. The literature also validates several key signalling molecules involved in water and energy homeostasis that have been identified in the AP at either the protein or the transcript level, such as angiotensinogen (*Agt*; Lewicki *et al.* 1978), apelin (*Apln*; Reaux *et al.* 2002), proenkephalin (Penk1; Rutherfurd & Gundlach, 1993; Engstrom *et al.* 2003), thyrotrophin-releasing hormone (Trh; Iwase *et al.* 1988), calcitonin/calcitonin-related polypeptide α (*Calca*; Fodor *et al.* 1994), cocaine and amphetamine related transcript (*Cartpt*; Zheng *et al.* 2002) and galanin (*Gal*; Krukoff *et al.* 1992; Koegler & Ritter, 1998; Fig. 2).

We then asked how gene expression within the AP changes in response to either physiological or pathological stress by comparison of the SD data with the DSD or the FSD data or by comparison between the SHR and WKY data. Intriguingly, we found that even in the same normalized experiment, a much larger number of genes were regulated in the AP in the pathological state compared with the physiological state. It is also worth noting that the range of fold-changes in the SHR data (compared with the WKY) was many times higher than that following either homeostatic challenge, with the highest fold-change being over 37-fold in the SHR. Regardless of whether this transcriptional overactivity is the cause of hypertension or the response by this structure to high blood pressure, we would predict that the large number of genes regulated here are important to the pathology and are therefore important targets for future study and treatment.

Following dehydration, corticotrophin-releasing hormone (*Crh*; 2.5-fold up), brain-derived neurotrophic factor (*Bdnf*; 2.4-fold up) and phosphatidylinositol 4-kinase (*Pi4k*; 3.5-fold down) are all regulated in the AP. The highest regulated gene following 48 h fasting is poorly annotated on the array; however, BLAST analysis reveals the probe sequence to belong to the major histocompatibility complex (MHC; 2.1-fold down). Within the SHR AP data, two of the highest regulated probe sets correspond to a single gene, the RT1-CE5 RT1 class I, locus CE5, a member of the MHC, whose expression is over 37-fold increased in the SHR for one probe set and 20-fold increased for a second probe set. Our array data also show that the transcript for epoxide hydrolase (*Ephx2*) is also upregulated in the hypertensive AP (Sellers *et al.* 2005). Also highly regulated in the SHR AP and represented by two probe sets is the gene for angiotensin II receptor-associated protein (*Agtrap*) that interacts with the carboxyl terminal of the angiotensin II type 1 receptor (present in the AP) to regulate its physiology (Daviet *et al.* 1999). The *Agtrap* probe sets are 3.3-fold and 9.5-fold upregulated.

We were interested to examine whether these three related signals (blood pressure, fasting and dehydration) regulated the same transcripts in the AP (S16; Fig. 1). In total, 10 transcripts (18% of D genes and 35% of F genes) are commonly regulated by both dehydration and fasting, and several interesting targets for future study are presented. For example, anoctamin 4 (*Ano4*), which is a putative calcium-activated chloride channel (Hartzell *et al.* 2009), and *homer3* associated with transient receptor potential cation channel (*Trpc1*; Beech, 2005; this channel is also Present on the array in the AP), both of which are downregulated in the AP in response to both dehydration and fasting. In total, six genes are commonly regulated by both dehydration and hypertension, including *Cd55* (upregulated in both states), suggested to inhibit the complement activation cascade and thus offer neuroprotection against hypoxic injury in neuronal cells (Wang *et al.* 2010), and *Rt1-Aw2* (downregulated following dehydration and upregulated in hypertension), a member of the rat MHC. Only two genes are regulated following fasting and hypertension (both downregulated following fasting and upregulated in the SHR), one of which is poorly annotated and the other, *Cos2*, is a negative regulator of Hedgehog target gene transcription involved in developmental pathways (Collier *et al.* 2004). Only the angiotensin receptor 1b (*Agtr1b*) is common to all three comparisons, which is downregulated following dehydration and fasting (1.7-fold and 1.9-fold, respectively) and upregulated in the hypertensive brain (2.4-fold). The fact that the expression of this gene does not appear significantly different between the SD and the WKY

rat (see above) implies that the expression profile across this experiment is not an artefact of strain-dependent gene expression.

In order to further investigate the putative physiological functions of the 315 genes regulated within the AP of the SHR, we first investigated whether any of the regulated genes converged on known QTL that might be important to the pathology of hypertension. Four genes, phosphorylase, glycogen, muscle (*Pygm*; represented by two probe sets), acetyl-coenzyme A acyltransferase 1 (*Acaa1*), tacykinin 1 and ATPase, Na^+/K^+ transporting, $\alpha 2$ polypeptide (*Atp1a2*), are annotated according to Affymetrix as either peaking or flanking known QTL for blood pressure (*Bp288*/*Bp331*), heart rate (QTL13) brain/spinal cord inflammation (QTL11) and serum renin concentration (QTL5), amongst others (Fig. 4). We then performed a GO analysis (S15; Fig. 5). Gene ontology analysis relies on the probability that a particular GO term is likely to appear in any given gene list above that of pure chance (on the entire data set). Using Genespring GX11, we revealed a list of 10 GO terms (adjusted $P < 0.05$), six of which refer to 'biological process' and the remaining four that refer to 'cellular component' (S15; Fig. 5). We are satisfied that one GO term enriched within our hypertensive gene list relate to 'regulation of blood pressure'. We also show that the genes in this list are related to various 'immune' duties. The hypertensive SHR has long been linked to immune dysfunction; the SHR displays a depression of T-lymphocyte function (Takeichi *et al.* 1981) and is responsive to immunosuppressive therapy with a drop in blood pressure (Khraibi *et al.* 1984). Recent work has also demonstrated that in addition to the brainstem expressing junctional adhesion molecule and excessive leukocyte binding, hypertension could be induced in a normotensive rat by evoking an immune response in the NTS, a structure with close anatomical and functional connections to the AP (Waki *et al.* 2007).

A perennial point for discussion with such data is that of causality. Is the regulation of these genes the cause of hypertension or are they being regulated in response to the hypertensive state? Rather than directly answering this conundrum, we have focused here on the functionality of these genes under the assumption that, regardless of the direction of causality, the targets presented here are part of a wider network of gene and protein elements that are disrupted in the disease state (see Nolan, 2007); the cure for hypertension is unlikely to be the result of a single gene or protein in a single tissue or organ. Within the hypertensive AP, we have identified specific groups of genes that are involved in either immune function or the regulation of blood pressure and converge on genetic loci involved with traits that underpin these functions and, as such, the genes within these lists make excellent targets for further functional study.

We have, for the first time, catalogued the transcriptome of the AP in two different strains of rats and identified those receptors and ion channels that are expressed in this CVO. We have also identified the transcriptional changes in the AP that result from the physiological stress of dehydration or fasting or from the pathological state of hypertension.

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