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Microarray analysis of the transcriptome of the subfornical organ in the rat: regulation by fluid and food deprivation

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Hindmarch C, Fry M, Yao ST, Smith PM, Murphy D, Ferguson AV. Microarray analysis of the transcriptome of the subfornical organ in the rat: regulation by fluid and food deprivation. *Am J Physiol Regul Integr Comp Physiol* 295: R1914–R1920, 2008. First published October 1, 2008; doi:10.1152/ajpregu.90560.2008.—We have employed microarray technology using Affymetrix 230 2.0 genome chips to initially catalog the transcriptome of the subfornical organ (SFO) under control conditions and to also evaluate the changes (common and differential) in gene expression induced by the challenges of fluid and food deprivation. We have identified a total of 17,293 genes tagged as present in one of our three experimental conditions, transcripts, which were then used as the basis for further filtering and statistical analysis. In total, the expression of 46 genes was changed in the SFO following dehydration compared with control animals (22 upregulated and 24 downregulated), with the largest change being the greater than fivefold increase in brain-derived neurotrophic factor (BDNF) expression, while significant changes in the expression of the calcium-sensing (upregulated) and apelin (downregulated) receptors were also reported. In contrast, food deprivation caused greater than twofold changes in a total of 687 transcripts (222 upregulated and 465 downregulated), including significant reductions in vasopressin, oxytocin, promelanin concentrating hormone, cocaine amphetamine-related transcript (CART), and the endothelin type B receptor, as well as increases in the expression of the $GABA_B$ receptor. Of these regulated transcripts, we identified 37 that are commonly regulated by fasting and dehydration, nine that were uniquely regulated by dehydration, and 650 that are uniquely regulated by fasting. We also found five transcripts that were differentially regulated by fasting and dehydration including BDNF and CART. In these studies we have for the first time described the transcriptome of the rat SFO and have in addition identified genes, the expression of which is significantly modified by either water or food deprivation.

dehydration; circumventricular organ; receptors

THE SUBFORNICAL ORGAN (SFO) is situated on the midline wall of the third ventricle in the dorsal region of the lamina terminalis. It is one of the sensory circumventricular organs (CVOs) and as such is distinct from the rest of the central nervous system (CNS) in its extensive vascular supply, which is derived from specialized capillaries lacking the normal blood-brain barrier (20). The SFO has also been shown to contain receptors for, and that respond to, a variety of different peripheral signals including regulatory peptides (e.g., angiotensin, amylin, ghrelin, atrial natriuretic peptide, and endothelin) (7, 32, 40, 48, 55), steroids (e.g., estradiol) (45), and ionic constituents of the extracellular environment (e.g., osmolarity, Ca^{2+}, Na^+) (3, 37, 44).

SFO neurons send dense efferent projections to important hypothalamic autonomic control centers including the paraventricular (PVN), supraoptic (SON), arcuate, median preoptic, and lateral hypothalamic nuclei of the hypothalamus (21, 28, 30, 33, 34). SFO neurons also receive afferent inputs primarily derived from lateral hypothalamus (29, 53), median preoptic nucleus (29), lateral parabrachial nucleus (22), and nucleus tractus solitarius (52, 57).

Despite the early view of the SFO as primarily an angiotensin sensor, 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 SFO in sensing immune (9, 15), metabolic (40, 42), and reproductive (5, 27, 50) signals have all now also been identified.

Although these past studies have relied upon both anatomical and physiological techniques to slowly piece together the functional roles of the SFO in autonomic regulation, recent technological advances in molecular biology provide new methods that permit a broader approach to the investigation of the integrative roles of structures such as the SFO in autonomic control. Microarray is a relatively new technology that allows the unbiased identification of both expressed transcripts in specific regions of the CNS as well as the effects of different physiological challenges on such transcript expression across the entire transcriptome.

The microarray technique relies upon the complementary hybridization of fluorescently labeled transcripts extracted from a sample to oligonucleotides that have been either immobilized or synthesized to a solid substrate. Differentially regulated genes are identified as those transcripts in which the signal ratio significantly exceeds the preset cutoff.

In the present study we have utilized Affymetrix whole rat genome chips to create the first complete description of the transcriptome of the SFO and to examine the effects of both fluid and food deprivation on the expression of this transcriptome.

MATERIALS AND METHODS

Animals. All experimental procedures were approved by the University of Bristol Ethical Review Committee and were carried out under government license in accord with the Animals (Scientific Procedures) Act 1986. Adult male Sprague-Dawley rats (10–12 wk

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old; Harlan Sera-lab, Loughborough, UK) were maintained in standardized temperature (22 ± 1°C), humidity (50 ± 5%), and diurnal conditions (10 h light and 14 h dark; lights on at 0700).

The control group of animals had access to both food and drinking water for the duration of the experiment. Three (dehydration group) or two (food deprivation group) days before tissue extraction, water bottles or food were removed, respectively, at 11 AM from separate groups of animals. Following 72 h of total water deprivation or 48 h of total food deprivation, the rats were euthanized (between the hours of 1100 and 1300) and the tissue was isolated and processed as described in *Tissue collection*. Control animals were also euthanized at the same time (between 1100 and 1300) each day.

Tissue collection. Rats were stunned and then decapitated with a small animal guillotine (Harvard Apparatus, Holliston, MA). The brain was rapidly removed from the cranium and placed in an ice-cold brain matrix (ASI Instruments, Warren, MI). One section of ~1-mm thickness was taken, and the SFO was carefully dissected on a bed of ice under a dissecting microscope (Leica). After isolation, the samples were rinsed in RNase-free PBS and then immediately placed in eppendorf tubes containing RNAlater (Ambion, Huntingdon, UK) and stored for no longer than 1 mo at −20°C before further processing. The above procedures were carried out in a RNase-free manner. A single operative carried out all dissections.

RNA extraction. Tissue was mechanically homogenized in QIAzol Lysis Reagent (Qiagen, Crawley, UK), and the aqueous phase was removed after centrifugation through a Phase Lock Gel column (Eppendorf, Cambridge, UK). Total RNA was purified with RNeasy Micro Kit MinElute Spin Columns (Qiagen) and eluted into 14 μl of RNase-free water.

Affymetrix GeneChip analysis. All RNA work was carried out in a Template-Tamer PCR workstation (Qbiogene, Cambridge, UK) to provide a nuclease-free environment. The quality of the RNA is essential to the overall success of gene expression analysis using microarray technology; thus stringent quality checks were carried out at all stages. The concentration and purity of the total RNA samples were first assessed by spectrophotometry (Jenway Genova Spectrophotometer; Jenway, Dunmow, UK). Only samples with a sufficiently high yield (>500 ng total RNA at a minimum concentration of 1.0 μg/μl) and purity (an A260:A280 ratio of close to 2) were further analyzed for quantity and integrity using the Agilent Bioanalyzer 2100 with RNA 6000 Nano Assay (Agilent Technologies, Stockport, UK). Samples that met the quality control criteria were used as templates for cRNA synthesis and biotin labeling, incorporating a single round of linear amplification, using the Ambion Message Amp II cRNA kit (Ambion). The quantity and size distribution of purified cRNA was assessed on the Agilent Bioanalyzer 2100 using RNA 6000 Nano Assay (Agilent) to ensure that the cRNA amplification was successful before proceeding to target fragmentation. This was achieved by incubation at 94°C for 35 min in fragmentation buffer containing (in mM) 40 Tris-Acetate (pH 8.1), 100 KOAc, and 30 MgOAc. The size distribution of the fragmented labeled transcripts was assessed on the Agilent Bioanalyzer 2100 using the RNA 6000 Nano Assay (Agilent). Samples were subsequently prepared for hybridization using the Affymetrix hybridization control kit (Affymetrix UK, High Wycombe, UK). All samples were hybridized to Rat Genome 230 plus 2.0 GeneChip arrays for 16 h in the Affymetrix GeneChip Hybridization Oven 6400 (Affymetrix UK). Following hybridization, the GeneChip arrays were stained and washed on the GeneChip Fluidics Station 400 (Affymetrix UK). Following hybridization of fragmented cRNA to GeneChip expression array probes, fluorescent signals were detected using the Affymetrix GeneChip Scanner 3000 (Affymetrix UK), which provides an image of the array and automatically stores high-resolution fluorescence intensity data. These data were initially documented using Affymetrix Microarray Suite software (MAS 5.0; Affymetrix UK), which generates an expression report file that lists the quality control parameters. All of these parameters were scrutinized to ensure that array data had reached the necessary quality standards (Scaling factor, <3-fold; average background values, 20–100; ~50–70% genes called present; ratio of 3′:5′ signal no more than 3). All raw data have been submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo; Accession No. GSE12978).

Microarray data analysis. Separate microarrays ($n = 5$) were probed using independently generated target from either control, dehydrated, or fasted rats. For each completely independent replicate, tissue from five rats was pooled for RNA extraction. Each comparison was independently normalized in accord with our standard protocol. Data were uploaded into GeneSpring software version 7.0 (Agilent Technologies) for normalization and high-level analysis as described in RESULTS. Expression values below 0.01 were set to 0.01. Data were normalized per chip and per gene to the median. All cutoff limits were set to 0.01, and all measurements were included in the normalization. Chip normalization was achieved by dividing each measurement by the 50th percentile of all measurements in that sample. The percentile was calculated with all raw measurements above 0.01. Gene normalization involved dividing each gene measurement by the median of its measurements in all samples. If the median of the raw values was below 0.01, then each measurement for that gene was divided by 0.01 if the numerator was above 0.01; otherwise the measurement was not considered in further analysis.

RESULTS

A comprehensive catalog of the genes expressed in the SFO. We first used GeneSpring to compile a list of genes called present (P) in all five independent control (C), dehydrated (D), or fasted (F) SFO experiments. All marginal, absent, or unknown calls were excluded. The number of transcripts listed as P in each condition, as well as the corresponding supporting supplementary tables, are listed in Table 1. The corresponding gene lists are transcriptome catalogs that, with a high degree of confidence, represent comprehensive descriptions of the RNA populations expressed in the SFO under control, dehydrated, and food-restricted conditions. We note that some transcripts that we might have expected to appear in our lists of expressed genes are not represented. For example, based on physiological analysis (51), one might have expected relaxin receptor expression to be identified in the SFO. There are a number of possible reasons for false negatives in our analysis. First, we employed stringent filtering criteria. Only genes called present in all replicates are included in our gene catalogs; some probe sets

Table 1. *Number of transcripts identified as being expressed in the rat SFO and significant changes as a consequence of dehydration or food restriction*

C-P	D-P	F-P	C + D + F-P	C vs. D > 1.5-fold	C vs. D > twofold	C vs. F > 1.5-fold	C vs. F > twofold
15,936 (S-1)	14,924 (S-2)	15,673 (S-3)	17,293	305 (S-4)	46 (S-5)	1,820 (S-6)	687 (S-7)

References in parentheses are to supplementary data tables. For independent control (C) vs. dehydrated (D) > 1.5-fold and vs. D > twofold and C vs. fasted (F) > 1.5-fold and vs. F > twofold, $P < 0.05$ (Benjamini-Hochberg multiple test correction). SFO, subfornical organ; P, present.

might have failed in some or all of the replicates, thus excluding them from analysis. Second, a gene might be absent from the array as in the case of the relaxin receptor LGR7 gene. Finally, a gene might be represented on the array, but without the correct annotation. Thus the reader is encouraged to further examine not only our supplementary data files (C-P, Supplementary Table 1; D-P, Supplementary Table 2; and F-P, Supplementary Table 3; all supplemental data can be found with the online version of this article) but also raw data that have been deposited with the NCBI GEO (see above) for further analysis.

We then used GeneSpring to combine the C-P, D-P, and F-P gene lists to produce a gene catalog that represents transcripts called present in all five experiments of either the C, D, or F conditions (C + D + F-P). Note that some of these genes, although, by definition, called present in all of the samples of one experimental condition, may well be called absent or marginal in some or all of the samples of the other condition (Table 1). These lists, which resulted in a combined total of 17,293 genes tagged as present in one of our three experimental conditions, were then used as the basis for further filtering and statistical analysis.

Changes in SFO steady-state RNA levels following dehydration. The C + D + F-P list was next filtered to identify genes that are putatively up- or downregulated by at least twofold following dehydration. Each of these new lists was used to statistically assess control versus dehydrated changes [Welch *t*-test; $P < 0.05$, with Benjamini-Hochberg (BH) multiple test correction]. Note that the false discovery rate of this protocol is ~5% of identified genes. Merging of the statistically filtered up- or downregulated gene lists (Table 1) gave us catalogs of transcripts that are significantly changed in abundance in the SFO following dehydration (Supplementary Tables 4 and 5). In total, 46 genes were changed in the SFO following dehydration compared with control animals (22 upregulated and 24 downregulated). The largest change identified in response to this dehydration was the more than fivefold increase in brain-derived neurotrophic factor (BDNF) expression (Fig. 1), a molecule that has been suggested to play significant roles in synaptic reorganization (23) and has been previously identified in the SFO (49). In addition, significant changes in the expression of the Ca-sensing receptors (CaSR; upregulated) and apelin receptors (downregulated) were also reported (Fig. 2). We have also compared this list of regulated genes with those that we have previously reported to be regulated by the same water deprivation stimulus in PVN, SON, and the neurointermediate lobe (NIL) (24). Four transcripts were commonly regulated in the SFO and the SON, one of which, a transcribed sequence, was also downregulated by dehydration in the NIL. The other three transcripts were upregulated in both the SFO and the SON following 3 days of dehydration (Table 2).

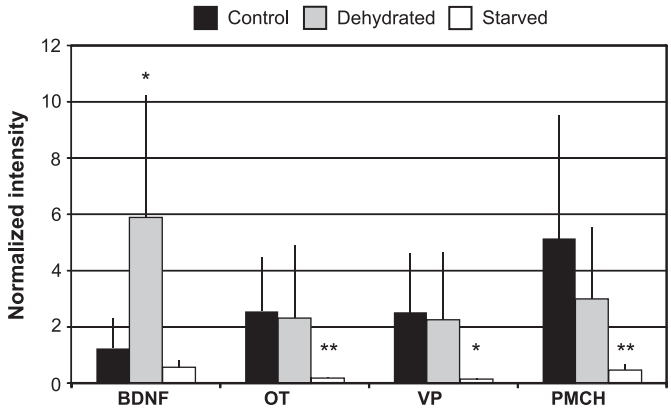

Fig. 1. Peptide expression (SD) in the subfornical organ (SFO): transcript expression of brain-derived neurotrophic factor (BDNF), oxytocin (OT), vasopressin (VP), and promelanin concentrating hormone (PMCH) in the SFO following starvation and dehydration. *P* values: *0.02 and **0.01 compared with control (*t*-test).

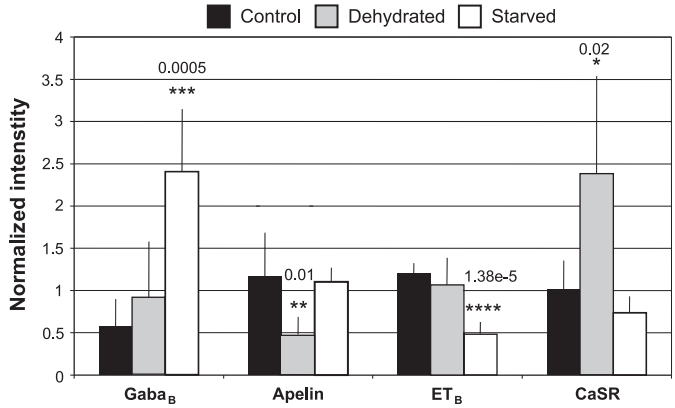

Fig. 2. Receptor expression in SFO: transcript expression of $GABA_B$, apelin receptor, endothelin type B receptor (ET_B), and Ca-sensing receptor (CaSR) in the SFO following starvation and dehydration. *P* values: *0.01, **0.02, ***0.0005, and ****$1.38e^{-5}$ compared with control (*t*-test).

Changes in SFO steady-state RNA levels following fasting. The same approach was adopted here as with the dehydrated data. The SFO-C + D + F-P comprehensive list was used to identify the genes that are putatively up- or downregulated as a result of fasting compared with control groups. This list was subjected to statistical assessment, resulting in lists of control versus fasted changes (Welch *t*-test; $P < 0.05$, with BH multiple test correction). Perhaps the most interesting aspect of this analysis was the observation that the lists produced reported a total of 687 transcripts that changed (compared with only 46 transcripts regulated by dehydration) between control and fasted animals (222 upregulated and 465 downregulated; Supplementary Table 7). This response to food deprivation included significant reductions in vasopressin (VP), oxytocin (OT), promelanin concentrating hormone (PMCH), cocaine amphetamine-related transcript (CART), and the endothelin type B receptor (ET_B), as well as increases in expression of the $GABA_B$ receptor (Figs. 1, 2, 4).

Coordinated changes in gene expression. Drinking behavior and food intake are closely related behaviors directed toward maintenance of homeostatic balance. Current evidence indicates that SFO plays a role in both behaviors (for review see Ref. 17) and that some hormones, including amylin (13, 41) and VP (25), may act at receptors in the SFO to simultaneously influence both water and food intake. Therefore, we further filtered the C + D + F-P list to identify transcripts that are coregulated with dehydration and food restriction.

Initially, we wanted to establish which of the transcripts that were regulated by either dehydration or fasting were commonly regulated by both conditions (Fig. 3). Of the 46 transcripts that are regulated by dehydration in the SFO, 37 are commonly regulated by fasting (Supplementary Table 12, b

Table 2. *Transcripts commonly regulated by dehydration and fasting in the SFO, NIL, and SON*

Affy ID	SFO CT vs. DH	SFO CT vs. ST	SON CT vs. DH	NIL CT vs. DH	Gene Description
1368359_a_at	2.565		3.577		VGF nerve growth factor inducible
1391923_at	2.036		2.426		Transcribed sequences
1392108_at	2.011		3.154	0.399	Transcribed sequences
1388116_at	0.448	0.365	0.489		collagen, type 1, α-1
1381522_at		0.235	0.46		UI-R-DC0-bzl-e-07-0-UI.s1 UI-R-DC0 Rattus norvegicus cDNA clone UI-R-DC0-bzl-e-07-0-UI 3′, mRNA sequence
1383447_at		0.414	2.184		Similar to ets variant gene 5 (LOC303828), mRNA

Supraoptic nucleus (SON) and neurointermediate lobe (NIL) data taken from Ref. 24. CT, control; DH, dehydrated; ST, starved.

and e), leaving just nine (Supplementary Table 12, a and d) that are uniquely regulated by dehydration and 650 (Supplementary Table 12, c and f) that are uniquely regulated by fasting.

Further Venn analysis was performed to identify transcripts that were regulated in different directions by dehydration and fasting. In total, five transcripts including BDNF and CART (Fig. 4), were observed in which signals increase relative to control (>1.5-fold) following fasting and decrease relative to control (>1.5-fold) following dehydration. This analysis did not identify any transcripts increased by dehydration and decreased by fasting.

Receptor expression within the SFO. In view of the significant literature describing the importance of a number of receptors found in the SFO that are involved in the regulation of body fluid and energy balance, we examined our microarray data from all three conditions to investigate patterns of receptor transcript expression. To first establish a list of receptors that are represented on the chips, the wildcard operator receptor was used to filter the 31,099 probe sets represented on the Affymetrix 230 2.0 microarray. Although this search returned a list of 1,963 features that appear on the chip (Supplementary Table 8) with the operator term receptor in their description and/or annotation, it is important to note that there will be both missing receptors (as a result of changing nomenclature) and false positives (as a result of the term receptor being used in the description, for example, in the case of the ligand). The C-P (Supplementary Table 9), D-P (Supplementary Table 10), and F-P (Supplementary Table 11) lists were next filtered to produce lists, which represent with a high degree of confidence a comprehensive list of receptors in which transcripts are expressed in the SFO (Table 3).

To identify receptor targets for further validation, the C-P list was filtered manually to ensure that the features represented are actual receptors. This list was then compared with the current literature describing receptor expression in SFO using functional and anatomical techniques. Our array data correlates well with the previous description of amylin (7), angiotensin (19, 32), calcitonin (46), CaSR (44), endothelin (35), and natriuretic peptide (36, 47) receptors in the SFO as illustrated in Fig. 5. In addition, these receptor-present lists identify a number of potentially important novel targets in SFO including adiponectin (AdipoR1, AdipoR2), apelin, leptin, endocannabinoid (CB1), and prolactin receptors, all of which are expressed at similar or higher relative levels than the angiotensin type 1 (AT_1) receptor as illustrated in Fig. 5. To determine whether the expression of transcripts encoding receptors was altered by dehydration or food restriction, the C + D + F-P (Table 1) list was next filtered to identify transcripts encoding receptors that are putatively up- or downregulated by at least twofold following dehydration. Each of these new lists was used to statisti-

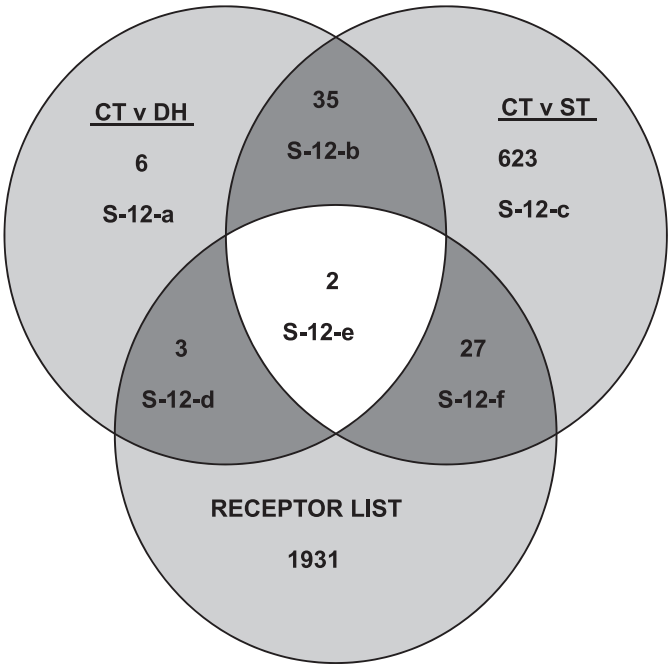

Fig. 3. This Venn diagram identifies transcripts that are commonly or uniquely regulated by starvation or dehydration and those that are identified by the wildcard operator of receptor. CT, control; DH, dehydrated; ST, starved.

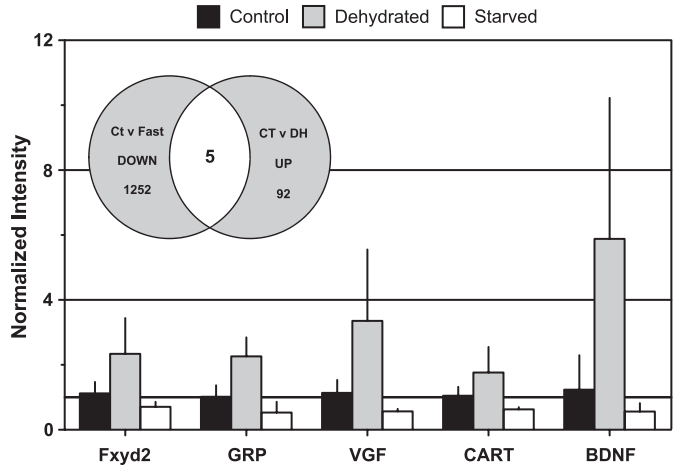

Fig. 4. Venn analysis (*inset*) identified transcripts that are regulated in different directions by dehydration and fasting (1.5-fold cutoff; $P < 0.05$; Welch's *t*-test, Benjamini and Hotchberg multiple testing correction applied). GRP, gastrin releasing peptide; CART, cocaine amphetamine-related transcript.

Table 3. *Numbers of transcripts on the Affymetrix 230 2.0 rat chip that contain the wildcard operator receptor in description and/or ontology*

Supplementary Table No.	Group	No. of Genes
8	All receptors	1,963
9	C-P	745
10	D-P	709
11	F-P	759

cally assess control versus dehydrated and control versus food-restricted changes (Welch *t*-test; $P < 0.05$, with BH multiple test correction). Note that the false discovery rate of this protocol is ~5% of identified genes. Merging of the statistically filtered up- or downregulated gene lists for both dehydration and fasting with the 1,963 features described as receptor gave us catalogs of transcripts encoding receptors that are significantly changed in abundance in the SFO following dehydration and food restriction. Of the 46 mRNAs that are significantly and robustly regulated by dehydration, a relatively large proportion (5 of 46) including the serotonin 5B, CaSR, and apelin receptor was tagged as receptor transcripts (Supplementary Table 12d). Two of these are also highly regulated by fasting (Supplementary Table 12e), which induced changes in only 29 receptor-tagged transcripts including $GABA_B$, ionotropic glutamate, endothelin B, and thyroid hormone-α receptors (Supplementary Table 12f). Proportional to the total number of significantly regulated genes, starvation regulates a relatively low number of receptor transcripts.

DISCUSSION

The SFO is a CVO that has recently been suggested to play important roles in the regulation of energy homeostasis (17, 18) in addition to its well-established physiological roles primarily in the regulation of fluid balance.

We have employed microarray technology using Affymetrix 230 2.0 genome chips to initially catalog the transcriptome of the SFO under control conditions and to also evaluate the changes (common and differential) in gene expression induced by the fluid and food deprivation. Although the statistical approaches we have taken for the analysis of our microarray data lead to a high degree of confidence in our findings, we also recognize the importance of validation of our findings using alternate technologies. However, the numbers of both new and regulated (by fluid or food deprivation) transcripts identified in these studies preclude any systematic analysis of all of these transcripts in the present study. Comparison of data obtained from our microarrays with current literature reporting binding sites, protein, and/or mRNA in SFO outlined below, however, does provide clear validation of expression and changes in a number of transcripts described in RESULTS.

The data obtained from our microarray analysis provides new information in a number of areas. Importantly, we have for the first time described a list of genes expressed in the SFO of control rats. Although our lists of normally expressed receptors are in accordance with many previous studies (AT_1, CaSR, ET_B, and natriuretic peptide A and B receptors), we have also identified the expression of a number of additional receptors including AdipoR1 and R2, CB1, leptin, prolactin, and thyroid hormone-α, all of which represent intriguing targets for more detailed future analysis. Similarly, our control-present lists provide confirmation of the expression of many ion channels (calcium, potassium, and sodium channels) and signaling molecules [angiotensinogen, BDNF, and pituitary adenylate cyclase-activating polypeptide (PACAP)], which have previously been reported in SFO, while again identifying the expression of some novel transcripts (VGF, CART, and aquaporin channels),

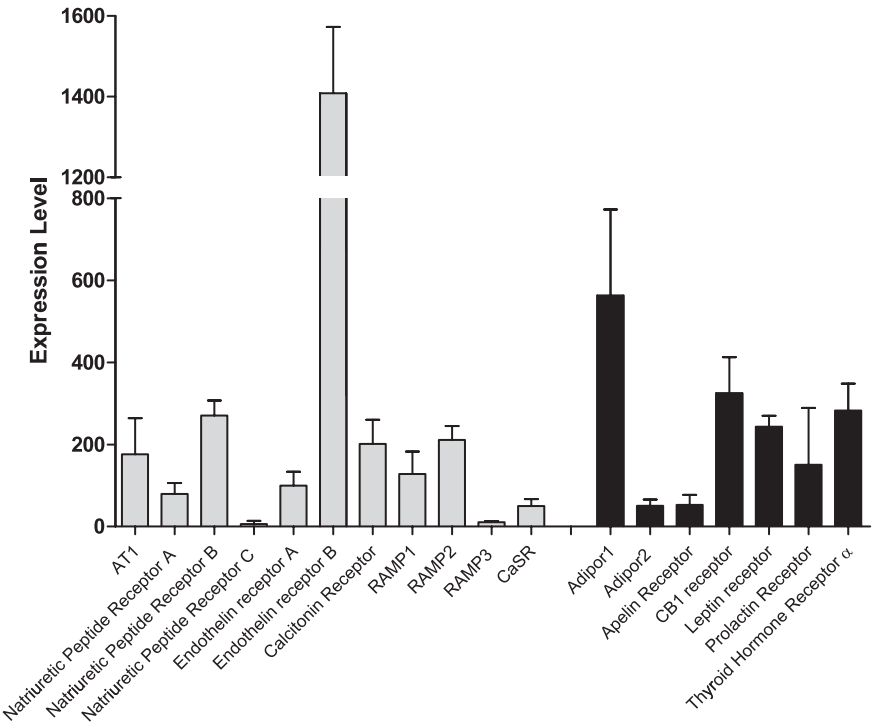

Fig. 5. Expression levels of both reported and novel receptors in the SFO. AT_1, angiotensin type 1 receptor; CB1, endocannabinoid; RAMP, receptor activity modifying proteins.

which may be of particular importance to elucidating the potential physiological roles of SFO in the regulation of ingestive behaviors.

The second focus of our microarray analysis of gene expression in SFO was to determine the effects of fluid and food deprivation on the transcriptome of this forebrain CVO. In these studies, we have used stringent statistical approaches (see MATERIALS AND METHODS) and initially only catalogued genes, the expression of which are modified twofold up or down by these challenges. Such analysis identifies relatively small numbers of transcripts that are either up (24)- or down (22)-regulated by dehydration. Interestingly, of the genes showing increased expression in response to fluid deprivation, the broader roles of both BDNF and VGF in the remodeling of neural circuits and connectivity (1, 2, 4, 43) are well established, suggesting the possibility that such changes within the SFO may represent a part of the response to dehydration. In addition, our data showing effects of fluid deprivation on BDNF are in accordance with previous work reporting increases in this neurotrophin following dehydration (49). Increases in expression of the CaSR following dehydration are also in accordance with the suggestion that CaSR in the SFO may play important roles in the regulation of body fluid composition, in particular the regulation of plasma Ca concentrations (56), and interactions of Ca-sensing systems with the renin-angiotensin system (54). Other studies evaluating the effects of dehydration on gene expression in SFO have reported increased angiotensin type 1A (AT_{1A}) receptor mRNA (6, 11) and PACAP mRNA and immunoreactivity (38). Although neither of these effects of dehydration is reported in our gene change lists, selective analysis of these specific genes does show clear changes in raw expression values in accordance with these previously reported changes (AT_{1A} control, 176.2 ± 39.4; dehydrated, 297.6 ± 58.2; PACAP control, 293.5 ± 54.23; and dehydrated, 509.2 ± 73.2), which do not meet the strict statistical cutoffs necessary to be included in our lists.

The emerging literature suggesting potential roles for the SFO in the regulation of energy balance led us to examine the effects of food deprivation for 48 h on gene expression in the SFO. Somewhat surprisingly, this challenge resulted in greater than twofold changes in over 10× more transcripts than were reported to change following dehydration, perhaps supporting the perspective that SFO may play an important role in the response to food deprivation. Although we are not aware of any previous studies examining the effects of food deprivation on gene expression in SFO, some intriguing observations emerge from the gene catalogs we have produced. Particularly interesting is the appearance in the top 10 downregulated genes of VP, OT, and PMCH, all of which are ~10-fold downregulated by food deprivation (Supplementary Table 7). We note that the latter two peptides have been suggested to play important roles in the regulation of food intake (14, 31, 39). Of interest in SFO genes downregulated by food deprivation are also our identification of the effects on CART and STAT3 (Supplementary Table 6), molecules previously shown to play important roles in the regulation of anorexigenic neural circuits originating in the arcuate nucleus (16, 26) and leptin signaling throughout the CNS (8, 10, 12), respectively. Similarly, significant increases in the expression of AdipoR2 and $GABA_B$ receptors, as well as the aquaporin 1 channel genes, were also observed in SFO following 48 h of food deprivation.

Perspectives

In these studies we have for the first time described the transcriptome of the rat SFO and have in addition identified genes, the expression of which are significantly modified by either water or food deprivation. It should be emphasized that the gene lists produced by these microarray studies are not in the first instance able to identify the potential functional significance of transcript modifications identified but do provide significant new data to drive hypothesis-oriented studies, which we hope will ultimately identify the causal relationships associated with the changes in expression we have described. Our gene lists (supplementary data) are freely available for download, and our raw data have been deposited in the GEO database at the NCBI. We encourage colleagues to further analyze our data, to form and test their own hypotheses, and to share information from such studies with the community as a whole. In this context, we hope that the databases provided here will stimulate new studies directed to furthering our understanding of the integrated roles of the SFO in the regulation of ingestive behaviors.

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