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Gene Expression Profiling in C57BL/6 Mice Treated with the Anorectic Drugs Sibutramine and Phendimetrazine and Their Mechanistic Implications

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Abstract

Recently, obesity has become a worldwide public health concern and the use of anorectic drugs has drastically increased. In this study, sibutramine and phendimetrazine, representative marketed anorectics, were repeatedly administered *per os* on a daily basis into C57BL/6 mice and the effects of these drugs on food intakes, body weight changes and gene expression profiles were monitored for up to following 7 days. Methamphetamine, which has a potent anorectic effect, was used as a positive control. Anorectic effects were sustained only for two days by phendimetrazine or methamphetamine, but for six days by sibutramine. The modulations of gene expressions in the hypothalamus and the striatum were investigated using microarrays on day 2 and day 7 post-administration, which corresponded to the anorectic period and a return of appetite respectively, for all three drugs tested. Differences in overall gene expression profiles in the stratum on day 2 for sibutramine and phendimetrazine seems to reflect difference between the two in terms of the onsets of drug tolerance. According to microarray findings, the Ankrd26 gene appears to have an important anorectic role, whereas the up-regulation of the olfaction system appeared to be involved in the drug tolerance of anorectics. The microarray data presented in this study demonstrates the usefulness of gene expression analysis for gathering information on the efficacy and safety of anorectic drugs.

Keywords: microarray, gene expression, anorectic drug, sibutramine, phendimetrazine, methamphetamine

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Introduction

Obesity has increased and become a worldwide public health problem. Most of the drugs used to treat obesity are appetite suppressants that stimulate the central nervous system (CNS), and these often have side effects, such as, tolerance, addiction, and cardiovascular problems.

Sibutramine is a representative anti-obesity drug that inhibits serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline reuptake (Stock, 1997). The mechanism underlying the anti-obesity effect of sibutramine is believed to involve satiety (Halford *et al.*, 1998) and the stimulation of thermogenesis (Conneley *et al.*, 1999; Liu *et al.*, 2002). In fact, pre-treatment with serotonin or norepinephrine antagonists can reduce the anorectic effect of sibutramine (Grignaschi *et al.*, 1999), which indicates that the anorectic effect of sibutramine is related to the inhibition of the reuptakes of norepinephrine (Balcioglu *et al.*, 2000) and serotonin (Buckett *et al.*, 1988; Gundlah *et al.*, 1997). Sibutramine is an approved drug for the long-term treatment for obesity, but has been reported to increase mean systolic and diastolic blood pressures and heart rate (Eric Colman, 2005).

Phendimetrazine has also been widely prescribed as an anorectic for the treatment of obesity, and has been reported to have properties similar to methamphetamine, which is known to suppress appetite by activating catecholaminergic neurotransmission (Seiden *et al.*, 1993; Chen *et al.*, 2001). Methamphetamine is known to primarily block dopamine transporter, which inhibits dopamine reuptake, indicating that dopamine up-regulation has an anorectic effect (Mackler *et al.*, 1993). Because phendimetrazine and methamphetamine stimulate the central nervous system to produce euphoria, probably via the activation of dopaminergic systems in the brain (Nailles *et al.*, 2003), these drugs are restricted to short-term use (a few weeks) and prominently labeled to warn against the risk of addiction.

However, although many anorectics are available, evidence is still lacking concerning their efficacies, safeties, and molecular mechanisms. Recently, cDNA microarray studies on gene expression profile changes by amphetamine have been reported (Noailles *et al.*, 2003; Yamamoto *et al.*, 2005), but no such report has been issued on other anorectics. In this study, we employed

microarray techniques to investigate the gene expression profiles of two representative anorectics, sibutramine and phendimetrazine and to compare these with that of methamphetamine in mouse brain. By comparing the gene expressional patterns of these drugs in different parts of mouse brain, we were able to identify genes whose expressions were specifically or commonly modulated by these drugs. We are in the belief that these identified genes would provide a molecular background and facilitate our understanding of the efficacies and safeties of anorectic drugs.

Methods

Animals

Mice were acclimated in the animal facility for seven days prior to commencing this study. Male C57BL/6 mice (20～25 g, Orient Bio, Korea) were housed individually in standard animal cages. All animal experiments were performed in accordance with a program accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Drugs and food intake

Methamphetamine (Sigma), phendimetrazine (Dreampharma, Korea) and sibutramine (Knoll AG, Ludwigshafen, Germany) were dissolved in deionized water. The effects of these anorectic drugs on food intake and body weight were monitored on a daily basis for seven days. 64 C57BL/6 mice (n=16 mice/group) were used for the experiments. Vehicle, methamphetamine (5 mg/kg), phendimetrazine (60 mg/kg) or sibutramine (5 mg/kg) were repeatedly administered *per os* to the animals on a daily basis. And deionized water was used as vehicle control. After the drug administrations, food and water were freely provided for following 18 hours before monitoring daily food intake and body weight changes. On day 2 and day 7 post-administration, respectively, a half of the animals in each group were sacrificed for brain isolation and mRNA extraction.

RNA isolation and DNA microarray

Total RNA was extracted from brain tissues using Trizol reagent (Invitrogen) in accordance with the manufacturer's instructions. Quantity and purity (260/280 ratio) of RNA were monitored using a ND-1000 UV/VIS spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Differential gene expressions were profiled using mouse genome survey array chips (Applied Biosystems, Foster City, CA) containing 60-mer oligonucleotide probes representing a set of 32,996 individual mouse genes and more than 1,000 control probes. Microarray experiments were performed according to the manufacturer's instructions. Digoxigenin (DIG)-UTP-labeled cRNA was generated from 5 μg of total RNA and amplified using chemiluminescent reverse transcription *in vitro* transcription (RT-IVT) labeling kits (Applied Biosystems). Briefly, each microarray was prehybridized in hybridization buffer with blocking reagent at 55°C for 1 h. DIG-labeled cRNA targets (10 μg) were fragmented to 100～400 bps and hybridized with each prehybridized microarray at 55°C for 16 h. Arrays were then washed with hybridization wash buffer followed by chemiluminescence rinse buffer. Chemiluminescent signals were generated by incubating arrays with anti-DIG alkaline phosphatase and chemiluminescence substrate. Images were collected for each microarray using a Model 1700 Chemiluminescent Microarray Analyzer (Applied Biosystems).

Analysis of microarray expression data

Signal intensities were imported into Avadis software (Strand and Stratagene, India). To minimize the effects of external variables, inter-array quantile normalization was performed. Average values of gene expression ratios obtained from three replicates were calculated. Differentially expressed genes (DEGs) were selected based on fold changes of ≥ 2 and a Welch's t-test probability value of ≤ 0.05. For further analysis, DEGs were categorized according to their biological functions using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification system (Applied Biosystems, https://panther.appliedbiosystems.com).

Results and Discussion

Effect of anorectics on food intakes and body weights changes in C57BL/6 mice

To identify genes related to the effects of anorectic drugs, we repeatedly administered single doses of phendimetrazine (60 mg/kg.), sibutramine (5 mg/kg), or methamphetamine (5 mg/kg, positive control) *per os* to C57BL/6 mice for up to 7 days and monitored daily changes in food intake and body weight over seven days. Fig. 1 shows the effects of the three drugs on food intake (Fig. 1A, B) and body weight (Fig. 1C, D) as comparing to vehicle only (Fig. 1A, C) and baseline (Fig. 1B, D), respectively. Rates of food intake were significantly suppressed in all three treated groups. The phendimetrazine and methamphetamine treated groups showed a maximum anorectic effect and weight loss on

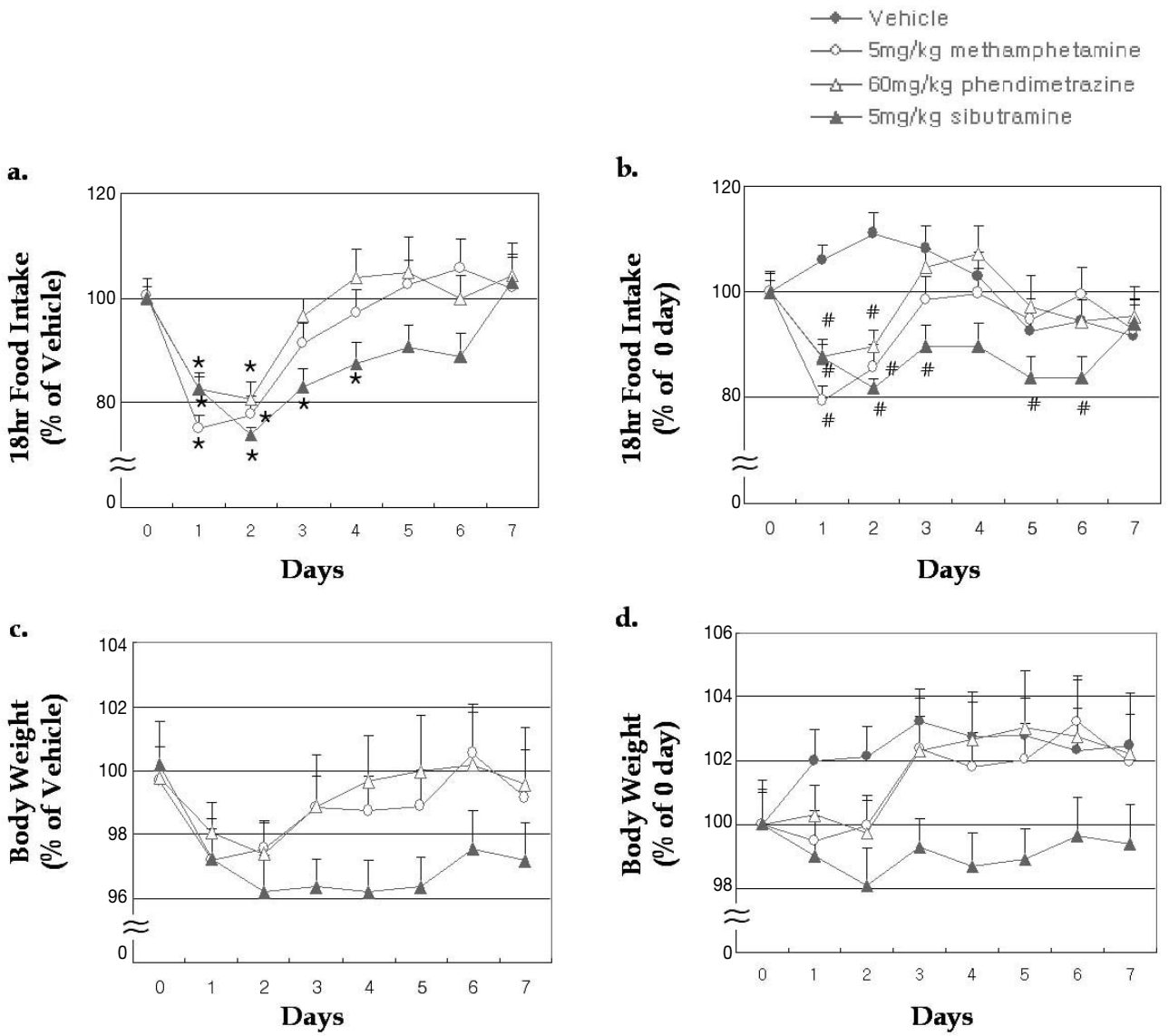

Fig. 1. Effects of anorectic drugs on food intake and body weight in C57BL/6 mice. The food intake rates as compared with vehicle treated controls (a) and baseline (b). Body weight changes versus the vehicle treated controls (c) and baseline (d). The lines above symbols represent standard errors. The asterisks (*) indicate significant differences between the vehicle controls and the three drug treatment groups and sharps (#) indicate significant differences between the drug treated groups and baseline ($p \leq 0.05$).

day 2 post-administration. After day 2, animals in these groups slowly recovered appetite and gained body weight to the control level. In contrast, the sibutramine-treated group, which exhibited the best anorectic effect and greatest weight loss at 2 days post-administration and drug effects were sustained up to day 6. However, on day 7 animals recovered their appetites in all treatment groups.

Phendimetrazine and methamphetamine show similar biochemical and behavioral properties (Crowin *et al*., 1987; Evans and Johanson *et al*., 1987; Jones and Holtzman 1994), and have both been reported to release dopamine and norepinephrine (Rothman *et al*., 2002). Moreover, the mechanism underlying the anorectic effect of these drugs has been attributed to the activation of catecholaminergic neurotransmission (Seiden, *et al*., 1993; Chen *et al*., 2001). On the other hand, sibutramine acts on serotonergic and noradrenergic pathways and has been demonstrated to effectively reduce body weight by reducing food intake and by modulating energy expenditure (Strack *et al*., 2002). These previous observations may explain why the anorectic effects of

sibutramine differed from those of methamphetamine and phendimetrazine in the present study. To better understand the modes of action of these anorectic drugs, we employed microarray techniques and investigated genes specifically and commonly modulated by these anorectic agents.

Genes differentially expressed in hypothalamus and striatum by sibutramine, phendimetrazine, and methamphetamine

Gene expression profiling was performed to examine the temporal effects of the three drugs on gene expression. Control of food intake and energy expenditure involves a complex network of neuropeptides, distributed throughout the central nervous system (CNS), but mainly in the hypothalamus (Schwartz *et al*., 2000; Saper *et al*., 2002). In the mammalian striatum, the basal ganglia nucleus subsumes many complex behaviors including feeding and sexual behavior (Alheid, G.F., 2003, Saint-Cyr, J.A., 2003). As described above, the three drugs showed a maximum effect on day 2 post-administration, though sibutramine had a sustained effect up to day 6. Based on these observations, total RNAs extracted from hypothalamus and striatum on days 2 and 7 were subjected to microarray analysis.

Table 1 shows the number of differentially expressed (fold change $\geq$2, p-value $\leq$0.05) genes on days 2 and 7 in the hypothalamus and striatum. Methamphetamine had the greatest effect on gene expression on day 2 in both hypothalamus and striatum, suggesting that it has a substantial effect on feeding behavior. Phendimetrazine induced a smaller number of differentially expressed genes on day 2 in hypothalamus than methamphetamine, which concurs with a previous observation that phendimetrazine has a lower anorectic effect than the other two drugs. However, phendimetrazine still modulated large number of genes in the striatum on day 2, which implies that it stimulates the central nervous system like methamphetamine. In the case of sibutramine, gene expression patterns differed slightly from those of phendimetrazine and methamphetamine. Sibutramine highly modulated gene expression only on day 2

Table 1. The number of differentially expressed genes (fold change $\geq$2, p-value $\leq$0.05) induced by the three anorectic drugs

	Hypothalamus			Striatum		
	Meth	Phen	Sibu	Meth	Phen	Sibu
Day 2	Up: 220 Down: 486 Total: 706	Up: 19 Down: 20 Total: 39	Up: 66 Down: 47 Total: 113	Up: 218 Down: 140 Total: 358	Up: 337 Down: 209 Total: 546	Up: 10 Down: 1 Total: 11
Day 7	Up: 10 Down: 214 Total: 224	Up: 7 Down: 4 Total: 11	Up: 0 Down: 1 Total: 1	Up: 176 Down: 35 Total: 211	Up: 130 Down: 22 Total: 152	Up: 139 Down: 49 Total: 188

Meth: methamphetamine, Phen: phendimetrazine, Sibu: sibutramine.

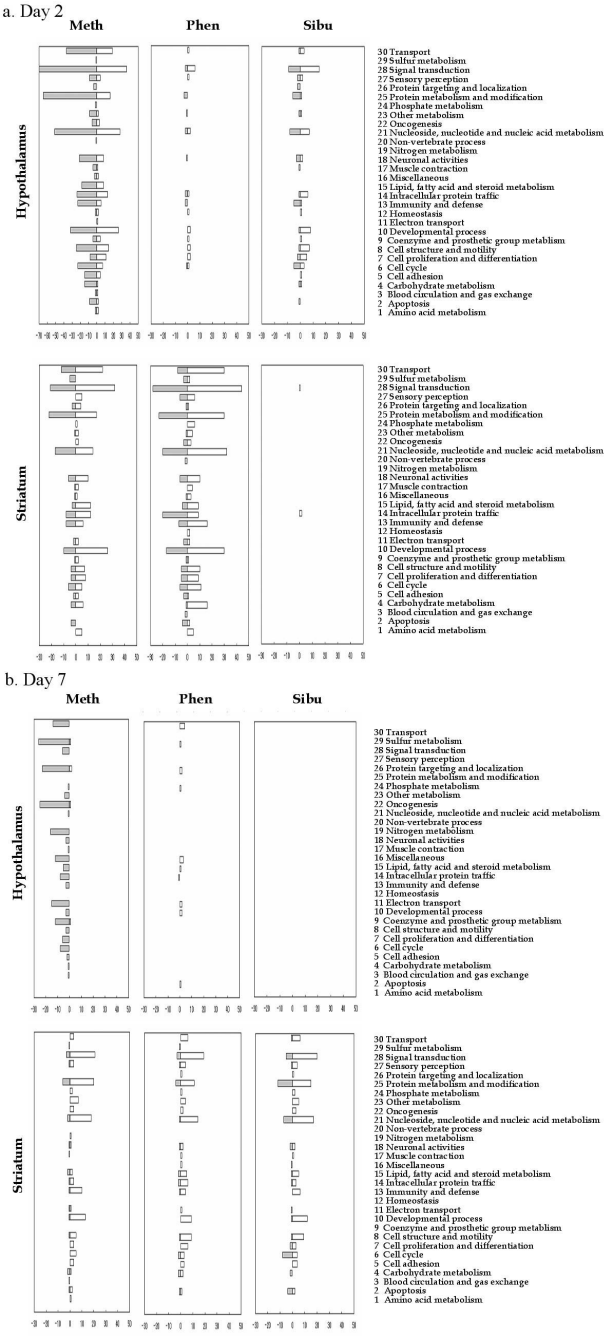

Fig. 2. Classification of genes differentially expressed in the hypothalamus and striatum on days 2 (a) and 7 (b) post-administration.

in the hypothalamus and did not showed significant gene modulation on day 2 in the striatum, which suggests its induction of anorexia differs mechanistically from those of phendimetrazine and methamphetamine. Nevertheless, all three anorectic drugs induced considerable differential gene expression in the striatum on day 7.

As described previously, all three drugs showed best efficacy on day 2, suggesting that genes modulated on day 2 in the hypothalamus are probably involved in anorectic effects. In fact, a large number of peptides and neurotransmitters in the hypothalamus affect energy balance. These include neuropeptide-Y (NPY), galanin, melanocyte stimulating hormone, and cocaine and amphetamine regulated transcript (CART) (Williams *et al*., 2000). Moreover, food deprivation is known to increase NPY mRNA in the hypothalamus of the goldfish and Coho and Chinook salmons (Silverstein *et al*., 1998;1999; Narnaware *et al*., 2000), and this effect can be reversed by re-feeding (Narnaware and Peter, 2001). Furthermore, CART was found to be a potent anorexigenic factor in mammals and to be most highly expressed in the hypothalamus (Gautvik *et al*., 1996). In addition, the actions of methamphetamine have been reported to be related to hypothalamic NPY and CART (Kuo 2003) and the anti-obesity actions of serotonin may be mediated by hypothalamic-NPY (Dryden *et al*., 1996).

A biological pathway analysis of gene expression profiles are considered as one of the most valuable tool in providing key information about the biological system and widely accepted by research community (Chung *et al*., 2007; Lee *et al*., 2008). To compare the biological pathways induced by the anorectic agents, we classified differentially expressed genes by biological process. As shown in Fig. 2, even though the numbers of differentially expressed genes varied for the three drugs, the genes modulated by phendimetrazine and methamphetamine showed similar biological process classifications on days 2 and 7 post-administration in the striatum. However sibutramine revealed a quite different biological pathway. The results of our pathway analysis strongly suggested that the biologic impact of sibutramine differs markedly from those of phendimetrazine and methamphetamine. To obtain detailed information on gene expression profiles, we further analyzed and compared individual genes modulated by the three drugs.

Analysis of gene expression profiles and safety considerations

To identify genes commonly and specifically expressed by the three drugs, Venn diagram analysis was employed (Fig. 3, 4). The numbers of genes commonly modulated by the three agents on day 2 was 16 in the hypothalamus and one in the striatum (Fig. 3). Since highest anorectic effects were observed on day 2 for all three drugs, commonly modulated genes on day 2, especially in the hypothalamus, might play a critical role in the anorectic effect of these drugs (Table 2).

Of the genes listed in Table 2, the ankyrin repeat domain 26 (Ankrd26) gene, which is up-regulated in the hypothalamus, revealed quite interesting information. The Ankrd26 gene is located at 10p12.1 in humans and

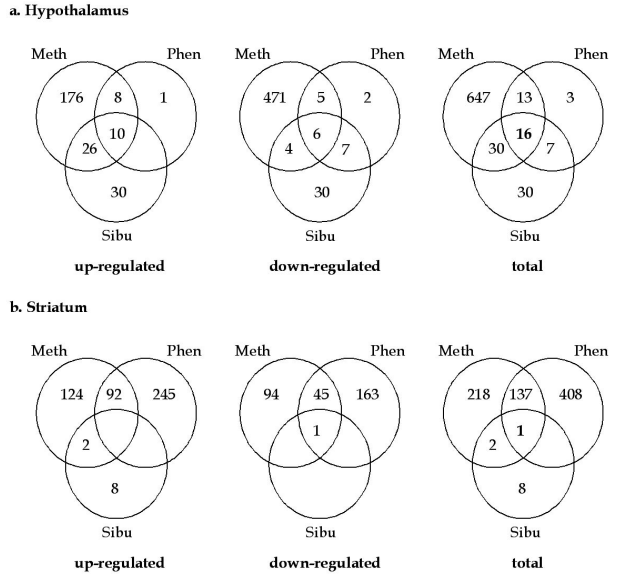

Fig. 3. Analysis of commonly modulated genes on day 2 post-administration in the hypothalamus (a) and striatum (b).

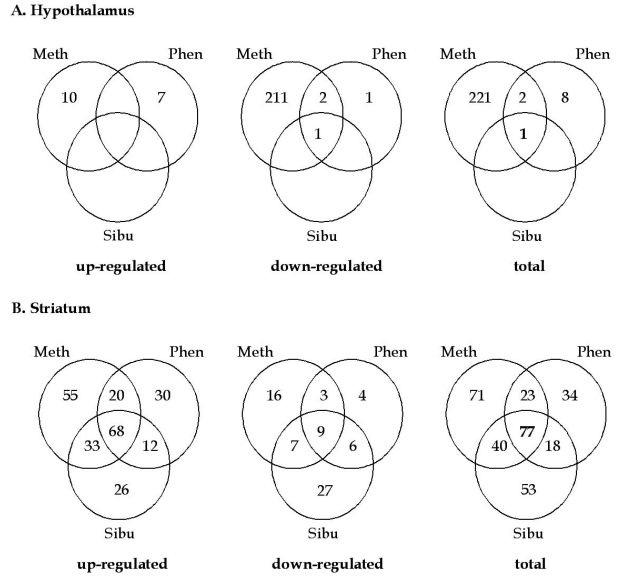

Fig. 4. Analysis of commonly modulated genes on day 7 post-administration in the hypothalamus (A) and striatum (B).

Table 2. List of genes commonly modulated in the striatum on day 2 post-administration

a. Hypothalamus

Gene_ID	Gene_symbol	Gene_name	Meth		Phen		Sib	
			p	Fold	p	Fold	p	Fold
mCG12278.3	Hip2	Huntingtin interacting protein 2	0.0000	−3.32	0.0002	−3.01	0.0016	−2.99
mCG5518.1	Reg3d	Regenerating islet-derived 3 delta	0.0038	−2.50	0.0008	−2.95	0.0177	−2.70
AK005238.1	Socs6	Suppressor of cytokine signaling 6	0.0021	2.31	0.0151	2.09	0.0087	2.08
mCG19763.1	LOC640437 Ankrd26	Ankyrin repeat domain 26	0.0028	2.41	0.0047	2.18	0.0068	2.30
mCG16888.2	Commd2	COMM domain containing 2	0.0073	2.52	0.0440	2.63	0.0070	2.39
mCG120273.1	LoC546387 Pcgf4	Polycomb group ring finger 4	0.0006	2.75	0.0379	2.16	0.0125	2.22
mCG141844	Pdzk2	PDZ domain containing 2	0.0000	3.32	0.0195	2.13	0.0094	2.48

b. Striatum

Gene_ID	Gene_symbol	Gene_name	Meth		Phen		Sib	
			p	Fold	p	Fold	p	Fold
AK041983.1	Stk22s1	Serine/threonine kinase 22 substrate 1	0.0078	−2.62	0.0002	−2.63	0.0226	−2.60

Genes without any known function are not shown.

at chromosome 6 (qF1) in the mouse (Hahn *et al.*, 2006). Furthermore, Ankrd26 is present in many normal tissues, but little information has been reported about function of this gene. However, recently, it was reported that Ankrd26 gene mutant mice develop extreme obesity, insulin resistance, and an increase in body size (Bera *et al.*, 2008), which suggests that Ankrd26 plays a critical function in controlling obesity. In accordance with this previous report, our microarray data showed that Ankrd26 was up-regulated on day 2 when anorectic effects were significant. Thus, our data supports the notion that Ankrd26 plays a key role in controlling obesity.

In the present study, only small numbers of genes were found to be differentially expressed in the striatum. Nevertheless, phendimetrazine and methamphetamine modulated the expressions of more genes than sibutramine, which again suggests that these two drugs share a common biological mechanism.

The numbers of genes commonly modulated by the three drugs on day 7 differed from that observed on day 2. On day 7, only one gene was commonly expressed in the hypothalamus, whereas 77 were commonly expressed in the striatum. This observation agrees well with the previous observation on food intake and body weight as described earlier. As described above, on day 7 animals in all three treatment groups recovered their appetites almost to the control level. This earlier observation was matched by microarray results, which revealed no significant change in gene expression on day 7 for phendimetrazine and sibutramine in the hypothalamus, which predominantly controls appetite. In contrast with the hypothalamus, microarray data revealed that a significant number of genes were expressionally modulated by the three drugs on day 7 in the striatum. By comparing lists of differentially expressed genes on day 7 in the striatum, we identified 77 genes commonly modulated by anorectic drugs (Table 3).

Of these 77 common genes, we found that six, which were highly up-regulated, were related to the olfaction system, namely, olfactory receptor 1022 (Olfr1022), olfactory receptor 1330 (Olfr1330), olfactory receptor 1134 (Olfr1134), vomeronasal 1 receptor I5 (V1ri5), vomeronasal 1 receptor A4 (V1ra4), and vomeronasal 1 receptor C2 (V1rc2). The olfaction system is a sensory dimension that plays an important role in food intake (Janowitz *et al.*, 1953; Le Magnen, 1981). It also has been reported that this system becomes more sensitive after fasting and less sensitive when satiated (Aime *et al.*, 2007; Apelbaum *et al.*, 2005; Pager *et al.*, 1972). Accordingly, in the present study, olfaction system up-regulation appeared to stimulate appetite in tested animals on day 7. Since the up-regulation of olfactory genes was greater on day 7 than on day 2, and increased olfaction system activity appeared to account for recovered appetite after prolonged treatment with anorectics. The striatum has been postulated to mediate the reinforcing properties of food and drugs of abuse. Furthermore, the release of dopamine in the striatum is

Table 3. List of genes commonly modulated in the striatum on day 7 post-administration

Gene_ID	Gene_symbol	Gene_name	Meth		Phen		Sib	
			p	Fold	p	Fold	p	Fold
mCG21421.2	Ugt8a	UDP galactosyltransferase 8A	0.0000	-19.86	0.0000	-59.57	0.0000	-60.34
mCG5518.1	Reg3d	Regenerating islet-derived 3 delta	0.0000	-5.06	0.0000	-3.73	0.0001	-2.13
mCG10496.2	Tanc1	Tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 1	0.0016	-4.44	0.0001	-7.34	0.0077	-3.03
mCG13143.2	Dnase1	Deoxyribonuclease I	0.0000	-4.26	0.0002	-5.33	0.0000	-5.74
mCG1876.2	Mdh2	Malate dehydrogenase 2, NAD (mitochondrial)	0.0000	-2.46	0.0001	-3.03	0.0000	-3.37
mCG3627.2	Gtf2a1	General transcription factor II A, 1	0.0169	2.18	0.0000	2.62	0.0001	2.51
mCG7000.1	Olfr1022	Olfactory receptor 1022	0.0238	2.36	0.0316	2.29	0.0197	2.14
mCG147992	LOC381955 AI326876	Expressed sequence AI326876	0.0028	2.42	0.0032	2.36	0.0000	2.56
mCG1049022	Aptx	Aprataxin	0.0004	2.46	0.0072	2.18	0.0067	2.43
mCG1037803.1	Ppp2ca	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	0.0001	2.47	0.0049	2.03	0.0011	2.66
mCG1042582.1	V1ri5	Vomeronasal 1 receptor, I5	0.0000	2.51	0.0002	2.06	0.0009	2.74
mCG12148.2	Ndufa4	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4	0.0000	2.53	0.0000	4.00	0.0000	3.36
mCG1037449	Cln3	Ceroid Lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeyer-Vogt disease)	0.0009	2.67	0.0085	2.28	0.0008	2.96
AK052337.1	Nr3c2	Nuclear receptor subfamily 3, group C, member 2	0.0032	2.89	0.0012	5.12	0.0023	4.29
mCG1038786.1	Olfr1330	Olfactor receptor 1330	0.0125	2.89	0.0001	3.33	0.0463	3.16
mCG1026173.1	Snapc1	Small nuclear RNA activation complex, polypeptide 1	0.0000	2.95	0.0000	2.14	0.0000	3.22
mCG3967.3	Hgfac	Hepatocyte growth factor activator	0.0048	3.00	0.0017	2.74	0.0021	2.13
mCG19062.3	Zfp207	Zinc finger protein 207	0.0000	3.03	0.0000	5.53	0.0000	4.99
mCG1031093.1	Igh-V	Immunoglobulin heavy chain variable regic	0.0002	3.07	0.0000	3.16	0.0015	3.94
mCG113912	Pde7b	Phosphodiesterase 7B	0.0001	3.11	0.0205	2.17	0.0001	2.56
mCG57121.2	Ndufab1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1	0.0000	3.16	0.0000	2.60	0.0000	3.19
mCG1042243	Spats1	Spermatogenesis associated, serine-rich 1	0.0000	3.21	0.0005	2.51	0.0000	2.81
mCG59442.1	LOC635999 LOC628161 Pin4	Protein (peptidyl-prolylcis/trans isomerase) NIMA-interacting, 4 (parvulin)	0.0000	3.28	0.0017	2.45	0.0001	3.51
mCG1048476	Stac	Src homology three (SH3) and cysteine rich domain	0.0000	3.37	0.0014	2.70	0.0000	3.17
mCG1030208.1	Capza1	Capping protein (actin filament) muscle Z-line, alpha 1	0.0000	3.39	0.0000	3.57	0.0000	4.04
mCG126582.1	Cldn5	Claudin 5	0.0000	3.59	0.0077	2.86	0.0092	2.66
mCG127499.1	LOC623273 Alms1	Alstrom syndrome 1 homolog (human)	0.0026	3.61	0.0114	3.46	0.0166	4.09
mCG121996.1	V1ra4	Vomeronasal 1 receptor, A4	0.0000	3.63	0.0001	2.96	0.0004	3.70
mCG115189	Il1f6	Interleukin 1 family, member 6	0.0008	3.79	0.0014	3.93	0.0012	3.46
mCG56416.2	Prox1	Prospero-related homeobox 1	0.0002	4.00	0.0074	2.58	0.0023	4.05
mCG16809.2	Rnd2	Rho family GTPase 2	0.0028	4.03	0.0003	9.85	0.0010	7.45
mCG1041193.1	Olfr1134	Olfactory receptor 1134	0.0100	4.10	0.0070	4.07	0.0285	3.65
mCG1042759.1	Mrps26	Mitochondrial ribosomal protein S26	0.0003	4.14	0.0082	2.69	0.0170	3.73
mCG130054	Psg17	Pregnancy specific glycoprotein 17	0.0000	4.72	0.0001	3.73	0.0000	3.06
mCG1032176	V1rc2	Vomeronasal 1 receptor, C2	0.0024	4.80	0.0010	4.07	0.0213	4.60
mCG141179.1	Snn	Stannin	0.0000	5.07	0.0000	15.12	0.0000	13.97
mCG140356	H2afz	H2A histone famile, member Z	0.0030	5.08	0.0001	5.39	0.0276	5.99
mCG3861.4	Map2k1	Mitogen activated protein kinase kinase 1	0.0000	6.08	0.0000	18.40	0.0000	16.50
NM_177148.3	Hrbl	HIV-1 Rev binding protein-like	0.0000	9.10	0.0000	24.09	0.0000	16.09

Genes without any known function are not shown.

known to be implicated in the mechanisms of drug addiction and neuroadaptation (Rompre and Wise, 1989; Robinson and Berridge, 1993). Accordingly, our striatal microarray data appears to reflect the importance of the striatum in drug tolerance.

In summary, we investigated gene expression profiles in the hypothalamus and striatum of C57BL/6 mice after administering sibutramine, phendimetrazine, or methamphetamine. We identified genes whose expressions were highly modulated by these drugs both in the hypothalamus and striatum. Analyses of gene expressions in these brain regions showed that sibutramine utilizes a biological pathway that differs from that utilized by phendimetrazine and methamphetamine. Further analysis of the genes commonly modulated by these three drugs resulted in the identification of Ankrd26, which probably accounts for the biological effects of these drugs. In addition, microarray data revealed the implication of the olfaction systems in the hypothalamus, which might explain why these anorectic drugs tend to lack efficacy after prolonged use. Our findings demonstrate that genes related to the olfaction system were significantly up-regulated at 7 days post-administration as compared with 2 days post-administration in the striatum, and since the appetites of drug-treated animals completely recovered to the control level on day 7, it would appear that olfactory genes play roles in the anorectic drug tolerance.

Our microarray experiments also revealed that sibutramine and the two other anorectics induced different gene expressions, and suggested that the biologic pathway induced by sibutramine differed from those induced by phendimetrazine and methamphetamine. In addition, sibutramine induced gene expressional changes on day 2 in striatum, a brain region implicated in drug tolerance. Our microarray data revealed that sibutramine also up-regulated genes related to olfaction system on day 7 in the striatum like phendimetrazine and methamphetamine. This observation suggests that sibutramine may invoke drug tolerance and that the prolonged use of sibutramine should be viewed with caution.

The microarray data presented in this study demonstrates that gene expression analysis can improve our understanding of the biological effect of anorectic drugs. Further studies involving microarray and clinical data are likely to provide profound information on drug efficacy and safety.

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Note

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