

Topiramate Normalizes Hippocampal NPY-LI in Flinders Sensitive Line ‘Depressed’ Rats and Upregulates NPY, Galanin, and CRH-LI in the Hypothalamus: Implications for Mood-Stabilizing and Weight Loss-Inducing Effects

Henriette Husum^{1,2}, Daniel van Kammen³, Evelien Termeer¹, Tom G Bolwig², Aleksander A Mathé^{*1}

¹Department of Physiology and Pharmacology, Division of Pharmacology, Institution of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; ²Neuropsychiatric Laboratory, Rigshospitalet, Copenhagen, Denmark; ³Johnson & Johnson Pharmaceutical Research and Development, Raritan, NJ, USA

Topiramate is currently used in the treatment of epilepsy, but this anticonvulsant drug has also been reported to exert mood-stabilizing effects and induce weight loss in patients. Neuropeptide Y (NPY) is abundantly and widely distributed in the mammalian central nervous system and centrally administered NPY markedly reduces pharmacologically induced seizures and induces antidepressant-like activity as well as feeding behavior. Two other peptides, galanin and corticotropin-releasing hormone (CRH), have also been proposed to play a modulatory role in mood, appetite, and seizure regulation. Consequently, we investigated the effects of single and repeated topiramate (10 days, once daily: 40 mg/kg i.p.) or vehicle treatment in ‘depressed’ flinders sensitive line (FSL) and control Flinders resistant line (FRL) rats on brain regional peptide concentrations of NPY, galanin, and CRH. The handling associated with repeated injections reduced hippocampal levels of NPY- and galanin-like immunoreactivities (LI) while NPY- and CRH-LI levels were increased in the hypothalamus, regardless of strain or treatment. In the hippocampus, concentrations of NPY-LI, galanin-LI, and CRH-LI were lower in FSL than FRL animals. Repeated topiramate treatment selectively normalized NPY-LI in this region in the FSL animals. In the hypothalamus, galanin-LI was reduced in FSL compared to FRL animals. Topiramate elevated the hypothalamic concentrations of NPY-LI, CRH-LI, and galanin-LI in both strains. Furthermore, topiramate elevated serum leptin but not corticosterone levels. The present findings show that topiramate has distinct effects on abnormal hippocampal levels of NPY, with possible implications for its anticonvulsant and mood-stabilizing effects. Furthermore, stimulating hypothalamic NPY-LI, CRH-LI and galanin-LI as well as serum leptin levels may be associated with the weight loss-inducing effects of topiramate.

Neuropsychopharmacology (2003) **28**, 1292–1299, advance online publication, 9 April 2003; doi:10.1038/sj.npp.1300178

Keywords: mood disorders; depression; corticosterone; leptin; HPA axis

INTRODUCTION

Topiramate is a novel neuroactive drug, currently indicated for the treatment of epilepsy. Multiple actions appear to account for its anticonvulsant effects, including inhibition of neuronal voltage-dependent sodium channels and L-type calcium-channel activation, enhancement of GABA_A receptor transmission and reduction of glutamatergic AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate), and kainate receptor neurotransmission (Shank *et al*, 2000). In

addition to anticonvulsant efficacy, other major clinical observations have been made. Specifically, topiramate has antimanic and anticycling effects in bipolar patients (Calabrese *et al*, 2001; McElroy *et al*, 2000). In addition, topiramate treatment has been associated with appetite suppression and weight loss in patients, in particular in overweight individuals (McElroy *et al*, 2000). The mechanisms of action underlying these phenomena are not known, and thus animal models may provide means for investigating these questions.

The Flinders sensitive line (FSL) rat has been developed by selective breeding for hypersensitivity to anticholinesterase agents and proposed as an animal model of depression because, like some depressed patients, they are more sensitive to cholinergic agonists (Russell *et al*, 1982; Janowsky *et al*, 1980; Overstreet *et al*, 1979). In addition, FSL rats display reduced body weight, reduced locomotor activity, increased rapid eye movement (REM) sleep,

*Correspondence: Professor AA Mathé, Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institutet, SE-17177 Stockholm, Sweden, Tel: +46 8 7287972, Fax: +46 8 331653, E-mail: aleksander.mathe@cns.ki.se

Received 11 October 2002; revised 14 January 2003; accepted 22 January 2003

Online publication: 3 February 2003 at <http://www.acnp.org/citations/Npp020303377/default.pdf>

cognitive deficits, and anhedonia in response to chronic mild stress (Yadid *et al*, 2000; Overstreet, 1993; Pucilowski *et al*, 1993), features that show resemblance to the symptom pattern of depression. We have previously shown that levels of neuropeptide Y (NPY), a highly conserved 36 amino-acid neuropeptide that is abundantly distributed in mammalian brain (Tatemoto *et al*, 1982), are reduced in the hippocampus of FSL rats (Husum *et al*, 2001; Jiménez Vasquez *et al*, 2000). Furthermore, in rodents and mice, exogenous NPY exerts antidepressant-like activity and modulates anxiety and stress behavior (Redrobe *et al*, 2002; Stogner and Holmes, 2000; Thorsell *et al*, 2000; Husum *et al*, 2000; Heilig *et al*, 1989). Most clinical studies have found reduced NPY concentrations in cerebrospinal fluid (CSF) and plasma from depressed patients and suicide attempters (Westrin *et al*, 1999; Nilsson *et al*, 1996; Hashimoto *et al*, 1996; Gjerris *et al*, 1992; Widerlöv *et al*, 1986). In one study, NPY-like immunoreactivities (NPY-LI) was increased in CSF from depressed patients following electroconvulsive therapy (ECT) (Mathé *et al*, 1996). Furthermore, treatment modalities used in the therapy of mood disorders, i.e. antidepressants and electroconvulsive stimulations (ECS, an experimental model of ECT), enhance NPYergic neurotransmission in rats (Jiménez Vasquez *et al*, 2000; Husum *et al*, 2000; Mathé *et al*, 1998; Stenfors *et al*, 1989; Widdowson and Halaris, 1989). These observations support the hypothesis that NPY plays a role in the pathogenesis as well as the alleviation of mood disorders.

Additional actions of NPY include stimulation of feeding behavior (Stanley *et al*, 1985) and potent reduction in duration and severity of pharmacologically induced seizures (Woldbye *et al*, 1997). An altered activity in the brain NPY system may therefore affect mood, appetite and seizure susceptibility. Two other peptides that have been implicated in these phenomena are corticotropin-releasing hormone (CRH) and galanin. The role of CRH in mood disorders has been extensively studied (for a review, see Holsboer, 2000) and was not the primary subject of this investigation. However, since CRH, in opposition to NPY, blocks feeding and has proconvulsant effects in rats (Brunson *et al*, 1998; Krahn *et al*, 1986), it was of interest to study it in this particular context. Galanin, in similarity to NPY, induces food ingestion in satiated rats and dampens the development of experimental epilepsy (Halford, 2001; Kokaia *et al*, 2001). Consequently, we hypothesized that the NPY, CRH, and galanin systems in the brain may be affected by topiramate treatment, which appears to influence the above-mentioned behaviors in humans.

The studies described in this paper were designed to gain insight into the effects of topiramate on regional brain levels of NPY, galanin, and CRH in an animal model of depression. In addition, serum leptin and corticosterone levels were assessed as measures of energy balance and hypothalamic–pituitary–adrenal (HPA) axis activity, respectively.

MATERIALS AND METHODS

Animals

Male FSL and Flinders resistant line (FRL) rats, 12–14 weeks old, from the rat colonies maintained at the animal facility

at the Karolinska Institutet were used. The animals were kept four per cage at a constant room temperature of $22 \pm 1^\circ\text{C}$ in a 12-h light/dark cycle (light on at 06:00) with free access to chow and tap water. The experiments were approved by the Stockholm's Ethical Committee for Protection of Animals and were conducted in accordance with the Karolinska Institutet's Guidelines for the Care and Use of Laboratory animals.

Treatments

The FSL and FRL rats were injected once daily (morning) with either topiramate (40 mg/kg i.p.) or vehicle (0.9% NaCl with added 3 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 7.0; 6.7 ml/kg). When previously administered to male Sprague–Dawley rats, this dose of topiramate yielded plasma concentrations of $44.05 \pm 9.57 \mu\text{M}$, which is within the anticonvulsant range of concentration (Reissmüller *et al*, 2000). Two experimental protocols were run: (1) a single injection and (2) a series of 10 injections. Body weights were recorded on the first and last day of treatment. Two hours after the last injection, the animals were decapitated and trunk blood was collected for serum sample preparation. The brains were quickly removed and dissected on ice into frontal cortex, striatum, occipital cortex, hippocampus, and hypothalamus and stored at -80°C , as were serum samples.

Peptide Extractions and Radioimmunoassay (RIA)

The tissue samples were homogenized, boiled twice in 1 M acetic acid and water, and centrifuged for 20 min at 1600g. The supernatants were lyophilized and reconstituted in phosphate buffer prior to RIA. RIA was performed using antibodies specific for NPY (RIN7180, Peninsula Laboratories, USA), CRH (a generous gift from Dr P Lowry (Linton and Lowry, 1986)), and galanin (GAL4, a generous gift from Dr E Theodorsson (Stenfors *et al*, 1989)). Within each experiment, all samples from a given brain region were analyzed in triplicate in the same run. Brain extracts and standard samples were preincubated for 48 h at 4°C with antibody. After addition of ^{125}I -labelled peptide (~ 6000 cpm per assay tube), all samples were incubated for another 24 h at 4°C (^{125}I -labelled NPY, galanin, and CRH were purchased from Amersham, Sweden). Free and antibody-bound radioligand were separated by addition of a sheep anti-rabbit antibody-coated Sepharose suspension (Pharmacia & Upjohn, Uppsala, Sweden). After 30 min of incubation at room temperature and centrifugation for 20 min at 1600g, 4°C , the supernatant was aspirated and discarded. The radioactivity in the pellets was measured in a γ -counter. The lower detection limits of the assays were 1.9 (NPY, galanin) and 31 pmol/l (CRH), and the intra-assay coefficients of variation were 5–7%.

Serum Samples

Serum samples were analyzed in duplicate for corticosterone (Diagnostics Products Corporation, USA) and leptin (DRG Diagnostics, DRG Instruments GmbH, Germany) by commercially available RIA kits. The lower detection limits of the assays were 20 and 0.5 ng/ml, respectively. The intra-assay coefficients of variation were 4–7%.

Table 1 Serum Concentrations of Corticosterone and Leptin

	Single treatment				Repeated treatment			
	FRL		FSL		FRL		FSL	
	Vehicle	Topiramate	Vehicle	Topiramate	Vehicle	Topiramate	Vehicle	Topiramate
Corticosterone	473 ± 171	365 ± 238	428 ± 153	451 ± 208	394 ± 188	549 ± 225	359 ± 217 [#]	260 ± 130 [#]
Leptin	4.74 ± 1.04	3.19 ± 0.86 ^{**}	2.91 ± 0.84 [#]	3.39 ± 0.52 [#]	1.92 ± 0.45	1.83 ± 0.75	1.29 ± 0.62 [#]	1.36 ± 0.44 [#]

Corticosterone and leptin levels were both lower in FSL compared to FRL rats ([#] $P < 0.05$). A single injection of topiramate (40 mg/kg i.p.) reduced serum leptin levels in the FRL rats to levels that were similar to those in FSL rats (^{**} $P < 0.01$ compared to vehicle-treated FRL). Data are presented as mean ng/ml ± SD, $n = 8$ per group.

Data Presentation and Statistical Analysis

In each experiment, overall group differences in variables were analyzed by two-way analysis of variance (ANOVA) with factors strain (FSL vs FRL) and treatment (topiramate vs vehicle). In addition, a three-way ANOVA was run to test the hypothesis that repeated handling/injections, compared to a single injection, may affect the measured variables. In case of a significant interaction, Tukey test was used for multiple comparison procedures. P -values < 0.05 were considered to be significant. Data are presented as mean ± SD.

RESULTS

Body Weight

Analysis of body weights on treatment day 1 showed that FSL (332 ± 51 g) weighed significantly less than FRL rats (361 ± 46 g, $F_{1,62} = 5.68$, $P < 0.05$, Student's t -test). Normalized values of body weight on treatment day 10 were computed with weight on treatment day 1 being set as 100%. Two-way ANOVA showed no significant effect of either strain or topiramate treatment on relative body weight gain (data not shown).

Serum Hormones

Corticosterone. Following a single injection, no strain or treatment effects on rat serum corticosterone concentrations (Table 1) were found. However, following repeated injections a strain difference became evident since corticosterone levels were significantly lower in FSL than FRL animals ($F_{1,25} = 5.0$, $P < 0.05$) while there was still no effect of topiramate.

Leptin. Following a single injection, leptin levels were higher in FRL compared to FSL rats ($F_{1,24} = 6.8$, $P < 0.05$) but there was no overall effect of treatment on leptin levels (Table 1). However, a significant strain × treatment interaction was found ($F_{1,24} = 11$, $P < 0.01$) since leptin levels were significantly higher in vehicle-treated FRL compared to vehicle-treated FSL rats ($P < 0.001$). Further, a single injection of topiramate selectively lowered leptin levels in FRL rats ($P < 0.01$), to levels similar to those in FSL rats. Following repeated injections, a strain effect was still found ($F_{1,25} = 6.6$, $P < 0.05$), but repeated topiramate had no significant effect.

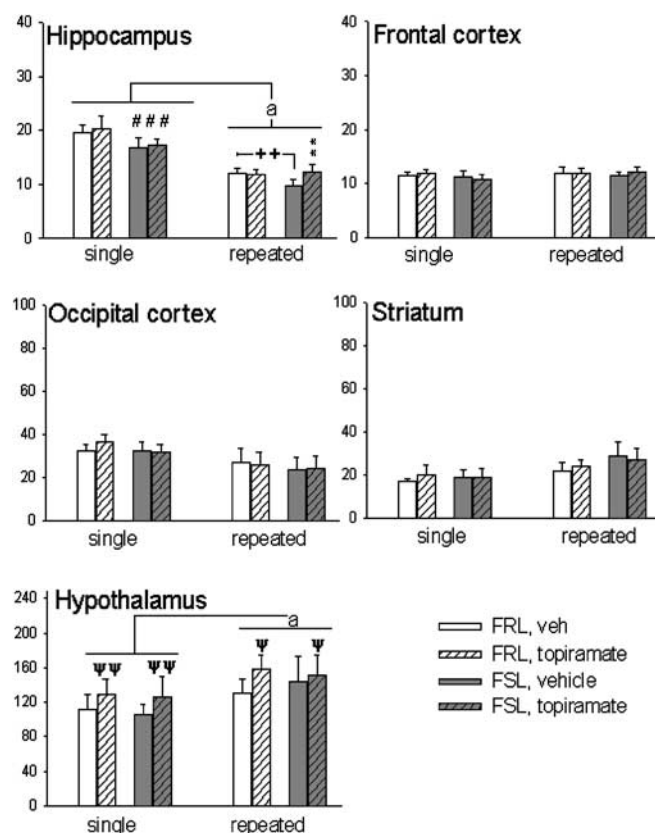


Figure 1 In the hippocampus, baseline NPY-LI levels were lower in FSL compared to FRL rats (^{###} $P < 0.001$, ⁺⁺ $P < 0.01$). Repeated topiramate treatment (40 mg/kg i.p.) normalized NPY-LI in the hippocampus of FSL compared to vehicle-treated FSL (^{**} $P < 0.01$). Interestingly, the handling associated with repeated injections markedly reduced NPY-LI in this region of both strains (^a $P < 0.001$). In the hypothalamus, topiramate increased NPY-LI in both strains (^ψ $P < 0.05$, ^{ψψ} $P < 0.01$). Again, there was an effect of repeated handling, which significantly increased hypothalamic NPY-LI levels in both strains (^a $P < 0.001$). Data are expressed as mean pmol NPY-LI/g wet weight tissue ± SD, $n = 8$ per group.

NPY

As shown in Figure 1, hippocampal levels of NPY-LI were lower in FSL than FRL animals following both single ($F_{1,28} = 22$, $P < 0.001$) and repeated injections ($F_{1,27} = 8.3$, $P < 0.01$). A single injection of topiramate had no effect on hippocampal NPY-LI. However, repeated topiramate

Table 2 Brain Regional Levels of CRH-LI

	Single treatment				Repeated treatment			
	FRL		FSL		FRL		FSL	
	Vehicle	Topiramate	Vehicle	Topiramate	Vehicle	Topiramate	Vehicle	Topiramate
Hippocampus	0.24 ± 0.05	0.27 ± 0.05	0.21 ± 0.04 [#]	0.22 ± 0.06 [#]	0.23 ± 0.04	0.21 ± 0.05	0.16 ± 0.02 [#]	0.19 ± 0.05 [#]
Hypothalamus	3.38 ± 0.86	3.55 ± 0.60	3.40 ± 0.89	3.59 ± 1.21	5.21 ± 0.84 ^a	6.45 ± 0.88 ^{††a}	4.54 ± 0.77 ^a	5.58 ± 1.69 ^{††a}

In the hippocampus, levels of CRH-LI were reduced in FSL compared to FRL rats following both single and repeated injections, regardless of treatment ([#] $P < 0.05$). In the hypothalamus no strain difference in CRH-LI was found following a single injection or repeated injections. Repeated topiramate (40 mg/kg i.p./10 days) significantly increased CRH-LI in this region (^{††} $P < 0.01$). Interestingly, the handling associated with repeated injections significantly increased hypothalamic CRH-LI levels, regardless of strain or treatment (^a $P < 0.001$). Data are expressed as mean pmol CRH-LI/g wet weight ± SD, $n = 8$ per group.

Table 3 Brain Regional Levels of Galanin-LI

	Single treatment				Repeated treatment			
	FRL		FSL		FRL		FSL	
	Vehicle	Topiramate	Vehicle	Topiramate	Vehicle	Topiramate	Vehicle	Topiramate
Hippocampus	13.4 ± 2.19	14.2 ± 2.33	12.4 ± 1.77	13.0 ± 2.21	9.94 ± 1.11 ^a	9.19 ± 1.69 ^a	8.35 ± 0.90 ^{†a}	8.51 ± 0.73 ^{†a}
Frontal cortex	4.69 ± 1.59	4.54 ± 1.52	4.56 ± 1.73	4.62 ± 1.30	2.70 ± 1.51	2.65 ± 0.89	2.31 ± 0.54	2.34 ± 1.85
Hypothalamus	115 ± 15.9	132 ± 14.5 [†]	107 ± 13.1 [#]	112 ± 14.4 ^{#,†}	129 ± 19.9	126 ± 24.8	116 ± 17.1 [#]	106 ± 12.8 [#]
Occipital cortex	2.71 ± 0.60	2.75 ± 0.63	2.60 ± 0.65	2.65 ± 0.56	2.68 ± 0.51	2.79 ± 0.66	2.47 ± 0.71	2.40 ± 0.56
Striatum	2.77 ± 0.76	2.81 ± 0.60	2.87 ± 1.09	3.45 ± 0.58	3.40 ± 0.99	4.32 ± 1.35	3.14 ± 1.10	3.12 ± 0.70

In the hippocampus, galanin-LI levels were lower in FSL compared to FRL rats ([#] $P < 0.05$). In addition, repeated injections significantly decreased galanin-LI in the hippocampus regardless of strain or treatment (^a $P < 0.001$). In the hypothalamus, galanin-LI was significantly lower in FSL rats following both single and repeated injections ([#] $P < 0.05$). A single injection of topiramate (40 mg/kg i.p.) increased galanin-LI in both strains ([†] $P < 0.05$). Data are expressed as mean pmol galanin-LI/g wet weight ± SD, $n = 8$ per group.

treatment significantly increased NPY-LI levels in the hippocampus ($F_{1,27} = 4.2$, $P < 0.05$) with a significant strain × treatment interaction ($F_{1,27} = 6.2$, $P < 0.05$). *Post hoc* analysis showed that NPY-LI levels were lower in vehicle-treated FSL compared to FRL rats ($P < 0.001$) and topiramate increased and normalized NPY-LI in the FSL strain ($P < 0.01$) but had no effect in the FRL rats. In addition, a significant effect of repeated handling was found as hippocampal NPY-LI levels were markedly reduced in animals subjected to repeated injections ($F_{2,56} = 367$, $P < 0.001$). This effect was independent of treatment, but there was a significant handling × strain interaction ($P < 0.01$) as the reduction in NPY-LI was larger in FRL than FSL rats.

In the hypothalamus, no significant strain difference in NPY-LI was found. Both single ($F_{1,28} = 8.4$, $P < 0.01$) and repeated topiramate injections ($F_{1,28} = 5.0$, $P < 0.05$) increased NPY-LI levels in the hypothalamus of both strains. Interestingly, repeated handling increased NPY-LI levels in the hypothalamus, regardless of strain or treatment ($F_{2,56} = 29.0$, $P < 0.001$).

In the striatum, occipital cortex, and frontal cortex, no strain differences in NPY-LI following either single or repeated injections were observed. Topiramate did not affect NPY-LI in these regions.

CRH-LI

CRH-LI (Table 2) was significantly reduced in the hippocampus of FSL rats following both single ($F_{1,27} = 5.2$, $P < 0.05$) and repeated injections ($F_{1,27} = 7.0$, $P < 0.05$)

regardless of treatment. Neither single nor repeated topiramate treatment affected hippocampal CRH-LI concentrations.

In the hypothalamus, no strain difference in CRH-LI was found following a single injection or repeated injections. Repeated topiramate injections ($F_{1,27} = 7.6$, $P = 0.01$), but not a single injection, increased CRH-LI in this region, in both FSL and FRL rats, compared to vehicle treatment. Repeated handling significantly increased hypothalamic CRH-LI levels, regardless of strain or treatment ($F_{2,56} = 60.3$, $P < 0.001$).

Galanin-LI

In FSL rats, hippocampal galanin-LI (Table 3) was significantly reduced compared to FRL rats following repeated injections ($F_{1,27} = 4.7$, $P < 0.05$) but not following a single injection. Neither single nor repeated topiramate injections affected hippocampal levels of galanin-LI. However, hippocampal galanin-LI levels were significantly reduced in animals subjected to repeated injections, regardless of strain or treatment ($F_{2,56} = 105$, $P < 0.001$).

In the hypothalamus, levels of galanin-LI were significantly reduced in FSL compared to FRL rats, following both single injection ($F_{1,27} = 7.3$, $P < 0.05$) and repeated injections ($F_{1,27} = 5.7$, $P < 0.05$). Single but not repeated injections of topiramate significantly increased hypothalamic galanin-LI in both strains ($F_{1,27} = 4.8$, $P < 0.05$).

In the striatum, frontal cortex, and occipital cortex, no strain differences in galanin-LI were observed following

either single or repeated injections. Topiramate also did not affect galanin-LI in these regions.

Discussion

The FSL rat displays abnormal behavior that resembles the symptom pattern of depression. One phenotypic trait of the FSL rat is that it displays increased immobility in the forced swim test, which can be reversed by chronic but not acute antidepressant treatment (Yadid *et al*, 2000; Overstreet, 1993; Schiller *et al*, 1992). In the present study, hippocampal NPY-LI concentrations were significantly lower in FSL 'depressed' rats compared to FRL control rats, in agreement with previous findings (Jiménez Vasquez *et al*, 2000; Husum *et al*, 2001). Interestingly, Fawn Hooded rats, another genetic model of depression, also have reduced hippocampal concentrations of NPY-LI (Mathé *et al*, 1998). In the present study the handling associated with repeated injections in itself markedly reduced hippocampal NPY-LI in particular in FRL but also in FSL animals. Furthermore, adult rats subjected to early life stress also have reduced levels of NPY-LI in the hippocampus (Husum and Mathé, 2002; Husum *et al*, 2002; Jiménez Vasquez *et al*, 2001). Cumulatively, these experiments clearly indicate that lowered hippocampal NPY, possibly resulting from exposure to repeated stress, is likely to be a common denominator of animal models of depression, and by extrapolation also a marker of depression in humans.

Repeated, but not acute, treatment with topiramate increased hippocampal NPY-LI selectively in the FSL rats. In these rats, NPY-LI levels were 'normalized', that is they were no longer different from the levels in FRL animals. ECS also increase NPY-LI in the hippocampus of FSL and normal rats (Jiménez Vasquez *et al*, 2000; Husum *et al*, 2000; Mathé *et al*, 1998; Stenfors *et al*, 1989). ECT has pronounced mood-normalizing and antipsychotic properties, but is also an anticonvulsant, as shown both in patients and in animal models of epilepsy (Nakajima *et al*, 2001; Post *et al*, 1984). Based on findings of increased hippocampal NPY release following kainic acid and ECS-induced seizures (Husum *et al*, 2000, 1998), attenuating effects of exogenous NPY against pharmacologically induced seizures (Woldbye, 1998; Woldbye *et al*, 1997, 1996), increased or even fatal vulnerability of NPY knock-down and knock-out mice to seizures (DePrato Primeaux *et al*, 2000; Baraban *et al*, 1997; Erickson *et al*, 1996), and altered brain synthesis of NPY in seizure-prone animals (Jinde *et al*, 1999; Amano *et al*, 1996; Sadamatsu *et al*, 1995), NPY has been suggested to act as an endogenous anticonvulsant (Bolwig *et al*, 1999). Consequently, the anticonvulsant properties of antiepileptic therapies, including ECT and topiramate treatment, may be attributable to a rise in hippocampal NPY-LI. This notion is supported by the present findings. It is not known whether voltage-sensitive sodium channels or L-type calcium channels are regulators of NPY synthesis. However, evoked, but not basal NPY release from rat hypothalamic slices is prevented by infusion of N-type, P/Q-type and Q-type calcium-channel inhibitors (King *et al*, 1999). Speculatively, L-type calcium channels may also exert this effect and thereby cause an accumulation of intracellular NPY-LI levels. In addition, rat brain levels of NPY-LI are regulated

by diazepam, an agonist for the modulatory site on the GABA_A receptor (Krysiak *et al*, 1999, 2000). Taken together, these reports suggest that topiramate may affect brain NPY-LI levels via its effect on L-type calcium channels and GABA_A receptors.

Topiramate is currently used in the treatment of epilepsy; however, there is also clinical evidence of mood-stabilizing properties of this drug. Thus, in one open study, half of the 44 treatment-refractory bipolar disorder patients showed moderate or marked improvement in symptoms when topiramate was added to existing therapy (Marcotte, 1998). In a recent study, bipolar patients showed improvement in mood following topiramate, in particular when patients exhibited manic symptoms at initiation of treatment (McElroy *et al*, 2000). There is also preliminary evidence of a beneficial effect of topiramate in treating post-traumatic stress disorder (Berlant, 2001). Most clinical studies have found reduced NPY concentrations in cerebrospinal fluid and plasma from depressed patients and suicide attempters (Westrin *et al*, 1999; Nilsson *et al*, 1996; Hashimoto *et al*, 1996; Gjerris *et al*, 1992; Widerlöv *et al*, 1986). Further, in rats as well as mice, NPY induces antidepressant-like behavior following central administration (Redrobe *et al*, 2002; Stogner and Holmes, 2000; Husum *et al*, 2000). The rise in hippocampal NPY-LI in FSL animals in the present study may therefore also be of importance to the proposed mood-stabilizing properties of topiramate. This is consistent with findings that other antidepressant treatment modalities also enhance hippocampal NPYergic neurotransmission (Husum *et al*, 2000; Widdowson and Halaris, 1989). In this regard it is noteworthy that there is a high comorbidity between epilepsy and mood disorders, and that the two types of disorders have been suggested to be related (Piazzini and Canger, 2001; Lambert and Robertson, 1999).

Hippocampal levels of CRH-LI were also reduced in FSL compared to FRL, but in contrast to NPY, unaffected by topiramate treatment. In a previous study, a modest non-significant reduction of CRH-LI was seen in the hippocampus of FSL rats (Owens *et al*, 1991). Intrahippocampal injections of CRH have been shown to enhance learning in mice (Radulovic *et al*, 1999) and, conversely, injections of antisense CRH gene oligonucleotides impaired memory retention in rats (Wu *et al*, 1997). The observed decrease of CRH-LI in the hippocampus may possibly reflect aspects of the impaired learning performance and cognitive deficits, which are previously described features of the FSL rat (Yadid *et al*, 2000; Overstreet, 1993).

Within the hypothalamus, NPY expressing neurons located in the arcuate nucleus project to the paraventricular nucleus, where NPY interacts with CRHergic neurons and thus modulates the reactivity and sensitivity of the HPA axis (Wahlestedt *et al*, 1987; Haas and George, 1987). In the present study, there was no strain difference in either hypothalamic CRH-LI or serum corticosterone. These findings are in agreement with a previous study where no baseline differences in hormone levels between FSL and FRL animals were found (Owens *et al*, 1991). However, repeated injections increased hypothalamic CRH-LI and NPY-LI levels in both rat strains while serum corticosterone was significantly decreased in FSL animals only. These changes may reflect the development of a blunted HPA axis stress

response in FSL but not FRL rats, following the stress associated with repeated injections (Holsboer, 2000). Interestingly, topiramate further increased CRH-LI and NPY-LI in the hypothalamus of both FSL and FRL animals. These findings are in agreement with a previous study in which hypothalamic NPY and CRH mRNA levels were increased by topiramate (York *et al*, 2000). Consistently with these findings, we have shown that early life stress-induced increase in hypothalamic NPY-LI levels is potentiated by lithium which however did not affect elevated CRH-LI levels in adult rats (Husum and Mathé, 2002). Further, antidepressant drugs also potentiate an elevated NPY gene transcription in the hypothalamus of rats subjected to stress (Makino *et al*, 2000). An upregulation of hypothalamic NPY-LI may therefore be of relevance to the therapeutic mechanism of action of mood-stabilizing drugs, while the regulation of CRH-LI appears to be less consistent, and may represent specific properties of topiramate.

Hypothalamic NPY and CRH influence food intake, since they elicit, respectively block, feeding in rats (Krahn *et al*, 1986). In one study, CRH was observed to block NPY-induced feeding in rats, suggesting that CRH overrides the effect of NPY in this behavioral aspect (Heinrichs *et al*, 1993). Consequently, it is possible that the observed increase in CRH-LI may be of significance to the long-term appetite-suppressive effects of topiramate reported to humans (McElroy *et al*, 2000).

Leptin is secreted from white adipose tissue and has a central role in coordinating responses and brown adipose tissue thermogenesis to maintain energy balance through effects on synthesis and secretion of NPY and other peptides in the CNS (Attele *et al*, 2002). Single but not repeated injections of topiramate reduced leptin in FRL rats, which became similar to levels in FSL, while topiramate had no effect on leptin levels in FSL rats. This is perhaps not surprising given the observation that topiramate affects food intake selectively in obesity-prone rats and only during the first 1–4 days of treatment (York *et al*, 2000). As also shown in the present study, a feature of the FSL rats is that they weigh less than FRL rats, likely reflecting a lower body fat mass, which could account for the lower leptin levels that were found in FSL rats.

We also assessed levels of galanin-LI in the brains of FSL and FRL rats. Lower galanin-LI levels were observed in the hippocampus of FSL animals compared to FRL, regardless of topiramate treatment. Interestingly, repeated handling in itself reduced hippocampal galanin levels in both strains. Previously, reduced galanin-LI levels and increased levels of galanin binding sites in the dorsal raphe nuclei of FSL rats were reported, suggestive of an association between the behavioral traits of the FSL rat and galanin hypofunction in this area (Bellido *et al*, 2002). In the hippocampal formation of Sprague–Dawley rats, the majority of galanin fibers were observed to colocalize with dopamine (Xu *et al*, 1998). Reserpine pretreatment depleted hippocampal levels of galanin, indicating a possible association between reserpine-induced behavior and low hippocampal levels of galanin (Xu *et al*, 1998). However, galanin has been reported to induce depressive-like behavior when injected centrally in the rat, a behavior that could be blocked by preadministration of a galanin antagonist (Weiss *et al*,

1998). Furthermore, in a previous study, galanin was not affected by repeated ECS (Stenfors *et al*, 1989). The functional significance of reduced hippocampal levels of galanin-LI in FSL rats is thus difficult to interpret, but may underlie cognitive aspects of the FSL rat phenotype that are unaffected by topiramate treatment.

In the hypothalamus, galanin-LI levels were significantly reduced in FSL rats, and single but not repeated injections of topiramate increased galanin-LI in this region of both strains. Galanin potently stimulates feeding in rats (Kyrkoulis *et al*, 1990) and, thus, the present observations are in line with reduced body weight of the FSL animals and may also be important to the reported appetite-suppressive effects of long-term topiramate treatment.

Taken together, the present findings are in line with our hypothesis that lowered hippocampal NPY levels may play a role in the pathogenesis of mood disorders and that one therapeutic mechanism of action of mood-stabilizing drugs, including topiramate, may be to normalize NPYergic neurotransmission in the hippocampus. Reduced levels of galanin in the hippocampus may also underlie aspects of the FSL rat 'depressed' phenotype, which are however unaffected by topiramate treatment.

The most striking differences in peptides were induced by repeated injections. This procedure (handling/injections) markedly reduced hippocampal levels of NPY and galanin and increased hypothalamic levels of CRH and NPY, results parallel to the previously reported findings of the effects of stress on these peptides. These findings also have relevance planning and interpretation of future experiments investigating these neuropeptides. Lastly, topiramate increased NPY-LI, galanin-LI, and CRH-LI in the hypothalamus. Although single or repeated topiramate treatment did not affect the body weight of either FSL or FRL animals in the present study, these changes may be of significance to the long-term weight loss-inducing effects of topiramate that are observed with humans.

ACKNOWLEDGEMENTS

This study was supported by the Johnson & Johnson Pharmaceutical Research and Development, Raritan, NJ, USA, the Swedish Medical Research Council, Grant 10414, the NAMI Research Institute-Stanley Foundation Bipolar Network, Ivan Nielsens Fond, and the Karolinska Institutet.

REFERENCES

- Amano S, Ihara N, Uemura S, Yokoyama M, Ikeda M, Serikawa T *et al* (1996). Development of a novel rat mutant with spontaneous limbic-like seizures. *Am J Pathol* **149**: 329–336.
- Attele AS, Shi ZQ, Yuan CS (2002). Leptin, gut and food intake. *Biochem Pharmacol* **63**: 1579–1783.
- Baraban SC, Hollopeter G, Erickson JC, Schwartzkroin PA, Palmiter RD (1997). Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. *J Neurosci* **17**: 8927–8936.
- Bellido I, Diaz-Cabiale Z, Jiménez Vasquez PA, Andbjør B, Mathé AA, Fuxe K (2002). Increased density of galanin binding sites in the dorsal raphe in a genetic rat model of depression. *Neurosci Lett* **317**: 101–105.
- Berlant JL (2001). Topiramate in posttraumatic stress disorder: preliminary clinical observations. *J Clin Psychiatry* **62**: 60–63.

- Bolwig TG, Woldbye DP, Mikkelsen JD (1999). Electroconvulsive therapy as an anticonvulsant: a possible role of neuropeptide Y (NPY). *JECT* 15: 93–101.
- Brunson KL, Schultz L, Baram TZ (1998). The *in vivo* proconvulsant effects of corticotropin releasing hormone in the developing rat are independent of ionotropic glutamate receptor activation. *Dev Brain Res* 111: 119–128.
- Calabrese JR, Keck PE, McElroy SL, Shelton MD (2001). A pilot study of topiramate as monotherapy in the treatment of acute mania. *J Clin Psychopharmacol* 21: 340–342.
- DePrato Primeaux S, Holmes PV, Martin RJ, Dean RG, Edwards GL (2000). Experimentally induced attenuation of neuropeptide-Y gene expression in transgenic mice increases mortality rate following seizures. *Neurosci Lett* 287: 61–64.
- Erickson JC, Clegg KE, Palmiter RD (1996). Sensitivity to leptin and susceptibility to seizure of mice lacking neuropeptide Y. *Nature* 381: 415–418.
- Gjerris A, Widerlöv E, Werdelin L, Ekman R (1992). Cerebrospinal fluid concentrations of neuropeptide Y in depressed patients and in controls. *J Psychiatry Neurosci* 17: 23–27.
- Haas DA, George SR (1987). Neuropeptide Y administration acutely increases hypothalamic corticotropin-releasing factor immunoreactivity: lack of effect in other rat brain regions. *Life Sci* 41: 2725–2731.
- Halford JC (2001). Pharmacology of appetite suppression: implication for the treatment of obesity. *Curr Drug Targets* 2: 353–370.
- Hashimoto H, Onishi H, Koide S, Kai T, Yamagami S (1996). Plasma neuropeptide Y in patients with major depressive disorder. *Neurosci Lett* 216: 57–60.
- Heilig M, Söderpalm B, Engel JA, Widerlöv E (1989). Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology* 98: 425–429.
- Heinrichs SC, Menzaghi F, Pich EM, Koob GF (1993). Corticotropin-releasing factor in paraventricular nucleus modulates feeding induced by neuropeptide Y. *Brain Res* 611: 18–24.
- Holsboer F (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology* 23: 477–501.
- Husum H, Jiménez Vazquez PA, Mathé AA (2001). Changed concentrations of tachykinins and neuropeptide Y in brain of a rat model of depression. Lithium treatment normalizes tachykinins. *Neuropsychopharmacology* 24: 183–191.
- Husum H, Mathé AA (2002). Early life stress affects concentrations of neuropeptide Y and corticotropin-releasing hormone in adult rat brain. Lithium treatment affects these changes. *Neuropsychopharmacology* 27: 757–764.
- Husum H, Mikkelsen JD, Hogg S, Mathé AA, Mork A (2000). Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. *Neuropharmacology* 39: 1463–1473.
- Husum H, Mikkelsen JD, Mørk A (1998). Extracellular levels of neuropeptide Y are markedly increased in the dorsal hippocampus of freely moving rats during kainic acid-induced seizures. *Brain Res* 781: 351–354.
- Husum H, Termeer E, Mathé AA, Cools AR, Ellenbroek BA (2002). Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonin-gene related peptide in adult rats. *Neuropharmacology* 42: 798–806.
- Janowsky DS, Risch C, Parker D, Huey L, Judd L (1980). Increased vulnerability to cholinergic stimulation in affective disorder patients. *Psychopharmacol Bull* 16: 29–31.
- Jiménez Vazquez PA, Mathé AA, Thomas JD, Riley EP, Ehlers CL (2001). Early maternal separation alters neuropeptide Y concentrations in selected brain regions in adult rats. *Dev Brain Res* 131: 149–152.
- Jiménez Vazquez PA, Overstreet DH, Mathé AA (2000). Neuropeptide Y in male and female brains of flinders sensitive line, a rat model of depression. Effects of electroconvulsive stimuli. *J Psychiatry Res* 34: 405–412.
- Jinde S, Masui A, Morinobu S, Takahashi Y, Tsunashima K, Noda A et al (1999). Elevated neuropeptide Y and corticotropin-releasing factor in the brain of a novel epileptic mutant rat: Noda epileptic rat. *Brain Res* 833: 286–290.
- King PJ, Widdowson PS, Doods HN, Williams G (1999). Regulation of neuropeptide Y release by neuropeptide Y receptor ligands and calcium channel antagonists in hypothalamic slices. *J Neurochem* 73: 641–646.
- Kokaia M, Holmberg K, Nanobashvili A, Xu ZQ, Kokaia Z, Lendahl U et al (2001). Suppressed kindling epileptogenesis in mice with ectopic overexpression of galanin. *Proc Natl Acad Sci* 98: 14006–14011.
- Krahn DD, Gosnell BA, Grace M, Levine AS (1986). CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Brain Res Bull* 17: 285–289.
- Krysiak R, Obuchowicz E, Herman ZS (1999). Diazepam and buspirone alter neuropeptide Y-like immunoreactivity in rat brain. *Neuropeptides* 33: 542–549.
- Krysiak R, Obuchowicz E, Herman ZS (2000). Conditioned fear-induced changes in neuropeptide Y-like immunoreactivity in rats: the effect of diazepam and buspirone. *Neuropeptides* 34: 148–157.
- Kyrkouli SE, Stanley BG, Seirafi RD, Leibowitz SF (1990). Stimulation of feeding by galanin: anatomical localization and behavioral specificity of this peptide's effects in the brain. *Peptides* 11: 995–1001.
- Lambert M, Robertson M (1999). Depression in epilepsy: etiology, phenomenology, and treatment. *Epilepsia* 40: S21–S47.
- Linton EA, Lowry PJ (1986). Comparison of a specific two-site immunoradiometric assay with radioimmunoassay for rat/human CRF-41. *Regul Pept* 14: 69–84.
- Makino S, Baker RA, Smith MA, Gold PW (2000). Differential regulation of neuropeptide Y mRNA expression in the arcuate nucleus and locus coeruleus by stress and antidepressants. *J Neuroendocrinology* 12: 387–395.
- Marcotte D (1998). Use of topiramate, a new anti-epileptic as a mood stabilizer. *J Affect Disord* 50: 245–251.
- Mathé AA, Jiménez PA, Theodorsson E, Stenfors C (1998). Neuropeptide Y, neurokinin A and neurotensin in brain regions of fawn hooded “depressed”, Wistar and Sprague–Dawley rats. Effects of electroconvulsive stimuli. *Prog Neuropsychopharmacol Biol Psychiatry* 22: 529–546.
- Mathé AA, Rudorfer MV, Stenfors C, Manji HK, Potter WZ, Theodorsson E (1996). Effects of electroconvulsive treatment on somatostatin, neuropeptide Y, endothelin, and neurokinin A concentrations in cerebrospinal fluid of depressed patients: a pilot study. *Depression* 3: 250–256.
- McElroy SL, Suppes T, Keck PE, Frye MA, Denicoff KD, Altshuler LL et al (2000). Open-label adjunctive topiramate in the treatment of bipolar disorders. *Biol Psychiatry* 47: 1025–1033.
- Nakajima T, Post RM, Pert A, Ketter TA, Weiss SR (2001). Perspectives on the mechanism of action of electroconvulsive therapy: anticonvulsant, peptidergic, and c-fos proto-oncogene effects. *Convulsive Ther* 5: 274–295.
- Nilsson C, Karlsson G, Blennow K, Heilig M, Ekman R (1996). Differences in the neuropeptide Y-like immunoreactivity of the plasma and platelets of human volunteers and depressed patients. *Peptides* 17: 359–362.
- Overstreet DH (1993). The Flinders sensitive line rats: a genetic animal model of depression. *Neurosci Biobehav Rev* 17: 51–68.
- Overstreet DH, Russell RW, Helps SC, Messenger M (1979). Selective breeding for sensitivity to the anticholinesterase DFP. *Psychopharmacology (Berl)* 65: 15–20.
- Owens MJ, Overstreet DH, Knight DL, Rezvani AH, Ritchie JC, Bissette G et al (1991). Alterations in the hypothalamic-pituitary-adrenal axis in a proposed animal model of depression

- with genetic muscarinic supersensitivity. *Neuropsychopharmacology* 4: 87–93.
- Piazzini A, Canger R (2001). Depression and anxiety in patients with epilepsy. *Epilepsia* 42: 29–31.
- Post RM, Putnam FW, Contel NR, Goldman B (1984). Electroconvulsive seizures inhibit amygdala kindling: implications for mechanisms of action in affective illness. *Epilepsia* 25: 234–239.
- Pucilowski O, Overstreet DH, Rezvani AH, Janowsky DS (1993). Chronic mild stress-induced anhedonia: greater effect in a genetic rat model of depression. *Physiol Behav* 54: 1215–1220.
- Radulovic J, Ruhmann A, Liepold T, Spiess J (1999). Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. *J Neurosci* 19: 5016–5025.
- Redrobe JP, Dumont Y, Fournier A, Quirion R (2002). The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. *Neuropsychopharmacology* 26: 615–624.
- Reissmüller E, Ebert U, Loscher W (2000). Anticonvulsant efficacy of topiramate in phenytoin-resistant kindled rats. *Epilepsia* 41: 372–379.
- Russell RW, Overstreet DH, Messenger M, Helps SC (1982). Selective breeding for sensitivity to DFP: generalization of effects beyond criterion variables. *Pharmacol Biochem Behav* 17: 885–891.
- Sadamatsu M, Kanai H, Masui A, Serikawa T, Yamada J, Sasa M *et al* (1995). Altered brain contents of neuropeptides in spontaneously epileptic rats (SER) and tremor rats with absence seizures. *Life Sci* 57: 523–531.
- Schiller GD, Pucilowski O, Wienicke C, Overstreet DH (1992). Immobility-reducing effects of antidepressants in a genetic animal model of depression. *Brain Res Bull* 28: 821–823.
- Shank RP, Gardocki JF, Streeter AJ, Maryanoff BE (2000). An overview of the preclinical aspects of topiramate: pharmacology, pharmacokinetics, and mechanism of action. *Epilepsia* 41(Suppl 1): S3–S9.
- Stanley BG, Chin AS, Leibowitz SF (1985). Feeding and drinking elicited by central injection of neuropeptide Y: evidence for a hypothalamic site(s) of action. *Brain Res Bull* 14: 521–524.
- Stenfors C, Theodorsson E, Mathé AA (1989). Effect of repeated electroconvulsive treatment on regional concentrations of tachykinins, neurotensin, vasoactive intestinal polypeptide, neuropeptide Y, and galanin in rat brain. *J Neurosci Res* 24: 445–450.
- Stogner KA, Holmes PV (2000). Neuropeptide-Y exerts antidepressant-like effects in the forced swim test in rats. *Eur J Pharmacol* 387: R9–R10.
- Tatemoto K, Carlquist M, Mutt V (1982). Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 296: 659–660.
- Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R *et al* (2000). Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. *Proc Natl Acad Sci USA* 97: 12852–12857.
- Wahlestedt C, Skagerberg G, Ekman R, Heilig M, Sundler F, Håkanson R (1987). Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. *Brain Res* 417: 33–38.
- Weiss JM, Bonsall RW, Demetrikopoulos MK, Emery MS, West CHK (1998). Galanin: a significant role in depression. *Ann NY Acad Sci* 863: 354–382.
- Westrin A, Ekman R, Traskman-Bendz L (1999). Alterations of corticotropin releasing hormone (CRH) and neuropeptide Y (NPY) plasma levels in mood disorder patients with a recent suicide attempt. *Eur Neuropsychopharmacol* 9: 205–211.
- Widdowson PS, Halaris AE (1989). Increased levels of neuropeptide Y—immunoreactivity in rat brain limbic structures following antidepressant treatment. *J Neurochem* 52: S77.
- Widerlöv E, Wahlestedt C, Håkanson R, Ekman R (1986). Altered brain neuropeptide function in psychiatric illnesses—with special emphasis on NPY and CRF in major depression. *Clin Neuropharmacol* 9: 572–574.
- Woldbye DP (1998). Antiepileptic effects of NPY on pentylenetetrazole seizures. *Regul Pept* 75–76: 279–282.
- Woldbye DP, Larsen PJ, Mikkelsen JD, Klemp K, Madsen TM, Bolwig TG (1997). Powerful inhibition of kainic acid induced seizures by neuropeptide Y via Y₅-like receptors. *Nat Med* 3: 761–764.
- Woldbye DP, Madsen TM, Larsen PJ, Mikkelsen JD, Bolwig TG (1996). Neuropeptide Y inhibits hippocampal seizures and wet dog shakes. *Brain Res* 737: 162–168.
- Wu HC, Chen KY, Lee WY, Lee EH (1997). Antisense oligonucleotides to corticotropin-releasing factor impair memory retention and increase exploration in rats. *Neuroscience* 78: 147–153.
- Xu ZQ, Shi TJ, Hokfelt T (1998). Galanin/GMAP- and NPY-like immunoreactivities in locus coeruleus and noradrenergic nerve terminals in the hippocampal formation and cortex with notes on the galanin-R1 and -R2 receptors. *J Comp Neurol* 392: 227–251.
- Yadid G, Nakash R, Deri I, Tamar G, Kinor N, Gispán I *et al* (2000). Elucidation of the neurobiology of depression: insights from a novel genetic animal model. *Prog Neurobiol* 62: 353–378.
- York DA, Singer L, Thomas S, Bray GA (2000). Effect of topiramate on body weight and body composition of Osborne-Mendel rats fed a high-fat diet: alterations in hormones, neuropeptide and uncoupling-protein mRNAs. *Nutrition* 16: 967–975.