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Effects of Sodium Valproate on Corticotropin-Releasing Factor Systems in Rat Brain

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We hypothesized that divalproex sodium, an anticonvulsant effective in the acute treatment of mania, may act upon neuropeptide systems that utilize corticotropin-releasing factor (CRF). Pharmacokinetic studies demonstrated that valproate has an apparent elimination half life of 17 minutes in rats after acute administration and that there is a nonlinear relationship between chronic dose and serum drug concentration. Acute valproate treatment neither altered plasma adrenocorticotropic hormone (ACTH) or corticosterone concentrations nor produced changes in CRF concentration in any of 10 brain regions examined. Subchronic treatment via SC-implanted osmotic minipumps (875 mg/kg/day × 7 days) resulted in decreased CRF concentrations in the median eminence and raphe nuclei. Moreover, CRF mRNA expression was decreased in the central nucleus of the amygdala (CeA) and paraventricular nucleus (PVN) of the hypothalamus. The benzodiazepine alprazolam, also a positive modulator of GABAergic function, similarly decreases CRF mRNA expression in the CeA. These results suggest that the mood stabilizing effects of valproic acid may be mediated in part by alterations in CRF neuronal activity.
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KEY WORDS: *Corticotropin-releasing factor (CRF); Valproic acid; Mood stabilizer; Bipolar disorder; Amygdala*

The precise mechanism(s) of action of clinically effective psychopharmacologic agents remains remarkably obscure and represents an area of intensive scrutiny. Corticotropin-releasing factor (CRF) neuronal systems are of particular interest, because they are known to play a seminal role in orchestrating the endocrine, autonomic, and behavioral responses to stress (Owens and Nemeroff 1991; Heit et al. 1997). Predisposing influences of stressors, abnormal stress responses, and/or symptoms of anxiety are characteristic of many psychiatric disorders. Furthermore, hyperactivity of CRF neuronal systems, including but not limited to parvocellular PVN neurons that regulate the hypothalamic-pituitary-adrenal (HPA) axis, is well documented in major depression and certain disorders (Plotsky et al 1998).

In the present study, the hypothesis was tested that divalproex sodium, effective for the treatment of bipolar disorder, may act upon neuronal systems that utilize CRF. Although studies of patients with euphoric mania have failed to detect alterations in cerebrospinal fluid (CSF) CRF concentrations (Berrettini et al 1987; Banki et al 1992), the hypothesis that valproate treatment affects CRF systems is worthy of consideration for several reasons. First, approximately 40% of manic patients exhibit dysphoric or mixed mania, and these patients exhibit HPA axis hyperactivity, presumably because of CRF hypersecretion. Second, valproate is well documented to be particularly effective in this subtype of bipolar

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disorder. In addition, benzodiazepines produce changes in CRF gene expression and peptide concentration (Owens et al. 1991; Imaki et al 1995; Skelton et al. 2000) that are opposite to those of acute or chronic stress. Through separate mechanisms, both benzodiazepines and valproate positively regulate GABAergic neurotransmission (Löscher 1993; Owens et al. 1997). Benzodiazepines have also been suggested to have therapeutic value as adjunctive treatment in active mania (Sachs 1996). In the current study, we have examined acute (90 min) and subchronic (7 days) effects of sodium valproate administration on brain CRF concentrations and other measures of pre- and postsynaptic CRF neuronal function.

METHODS

Animals and Drug Administration

Male, Sprague–Dawley rats weighing 325 to 375 g were used for all of these studies. Divalproex sodium is an equimolar mixture of sodium valproate and valproic acid. At physiological pH, the valproate moiety is the active agent. For all studies, sodium valproate (2-propyl pentanoic acid-Na salt; Sigma, St. Louis, MO) was utilized. Sodium valproate was dissolved in vehicle (water) and injected subcutaneously in a volume of 1 ml/kg for the pharmacokinetic and acute CRF studies, 20 to 90 minutes before decapitation. For subchronic administration studies, one or two osmotic minipumps (Alzet model 2ML1, Alza Corp.) per rat were filled with an aqueous solution of sodium valproate and implanted in the subcutaneous, intrascapular region. Rats were killed by decapitation as specifically approved by the Emory University IACUC under NIH guidelines for the care and use of laboratory animals, and trunk blood and brains were rapidly collected. Brains were frozen on dry ice and stored at −80°C.

Serum Valproate Determination

For the quantitative determination of this drug, we utilized the assay available on the Abbott AxSYM™ immunoassay analyzer. This assay employs fluorescence polarization immunoassay (FPIA) technology. The system requires 150 μl of sample and uses a six-level calibration curve. Three levels of quality control are performed daily on the system. For the assays performed as part of this study, the interassay coefficients of variation (CV) were 6.0, 5.1, and 4.7% at levels of 37.5, 75.0, and 125.0 μg/ml, respectively. The sensitivity of the assay is 0.70 μg/ml as defined by the manufacturer and verified in our laboratory. 3-keto-valproic acid, the major metabolite of valproic acid exhibits <10% crossreactivity. Minor metabolites of valproic acid (3-hydroxy-valproic acid, 4-hydroxy-valproic acid, 4-en-valproic acid, 2-propyl-glutarate, and 5-hydroxy-valproic acid) tested at high concentrations exhibited crossreactivity below the assay sensitivity. This assay (as configured by the manufacturer) measures total valproic acid.

CRF Radioimmunoassay

Brains were thawed on a chilled glass plate and dissected into brain regions according to anatomic landmarks (Paxinos and Watson 1986). CRF was extracted from tissue dissections using 1 mol/l HCl with protease inhibitors as previously described in detail (Ladd et al 1996). CRF content was determined in duplicate by radioimmunoassay, using a rat/human CRF antiserum obtained from Peninsula Laboratories (final dilution 1:23,000). Synthetic peptides were used for the CRF standard (Bachem), and [^{125}I]-Tyr^{0}-r/hCRF as the tracer (NEN Dupont). The limit of sensitivity was 2.5 pg/tube; with few exceptions, the sample tubes contained at least 10 pg CRF. Data were normalized with respect to protein content as determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

In Situ Hybridization Histochemistry (ISHH)

The rat prepro-CRF plasmid (provided by K. Mayo, Northwestern University, Evanson, IL) includes the first 1195 bp of the CRF sequence (Genbank X03036) cloned into pGEM-4Z vector. The rat CRF_1 receptor plasmid (W. Vale, Salk Institute) includes bases 217-1412 of the CRF_1 sequence (Genbank L24096) cloned into pBluescript II. Antisense templates were generated by restriction endonuclease digestion with PvuII or BsaHI, and riboprobes synthesized using an Ambion Maxiscript kit with SP6 or T3 polymerases, respectively. The CRF transcription reaction included a threefold isotopic dilution of [^{35}S]-UTP (1250 Ci/mmol, NEN Dupont); whereas, specific activity of the CRF_1 receptor riboprobe was increased by including 100% ^{33}P-CTP (2000–4000 Ci/mmol). Probes were purified by ethanol precipitation. Sense strand probes of comparable specific activity were also prepared (CRF: FspI/T7; CRF_1: HindIII/T7), neither of which yielded any detectable labeling in the brain regions analyzed.

Brains were coronally sectioned at 20 μm (every fifth section sampled per assay), thaw-mounted onto Fisher Superfrost Plus slides, and stored at −80°C. On the day of the assay, sections were brought to room temperature, postfixed in 4% paraformaldehyde, and washed twice briefly in phosphate-buffered saline (Sigma). Remaining steps were carried out according to the method of Simmons et al. (1989), using the recommended hybridization conditions of 60°C moist heat for 16 to 20 hours and a final stringency wash of 0.1X SSC/0.1% DTT, for 30 min at 60°C. Between 1.4 and 2 × 10^6 cpms

of probe solution were used per slide. After the ISHH procedure, slides were apposed to Kodak Biomax film for a time period empirically chosen based on the signal intensity for each region of interest (ROI). Optimal exposure times produced easily identifiable nuclei, with rare or no patches of saturation.

Image Analysis

Film autoradiograms were digitized using a Dage-MTI CCD-72 (Michigan City, IN) video camera and analyzed using Scion Image. Pixel density was determined in brain ROIs (PVN, CeA, BNST), using circled areas of consistent size and shape. The PVN area included both parvocellular and magnocellular portions of this nucleus; it was impossible to select only the parvocellular PVN without a detailed histological analysis. The initial BNST measurement included both dorsolateral and ventral portions of this nucleus; a second analysis of the dorsolateral BNST alone was conducted post hoc, after obtaining a positive result in the functionally related CeA (Alheid and Heimer 1988). Background density was determined in an adjacent region of each section. Density values were converted to tissue equivalent activity using [^{14}C] standard curves (Amersham, American Radiolabeled Chemicals) acquired under the same conditions. Last, the three (PVN, BNST) or four (CeA) highest values from each brain were averaged.

ACTH and Corticosterone Assays

Plasma concentrations of corticotropin (ACTH) were determined using a two-site immunoradiometric assay (Nichols). Serum concentrations of corticosterone were determined using a commercial radioimmunoassay kit (ICN Biochemicals). Intra- and interassay variability for these assays were less than 8%.

RESULTS

Valproate Dosing and Pharmacokinetics

To characterize the basic kinetics of valproate elimination, rats were injected SC with 1, 10, or 100 mg/kg sodium valproate and killed after 20, 40, 60, or 90 min (Figure 1). The results indicate that valproate has an apparent elimination half life of approximately 17 minutes with apparent first-order kinetics.

In the acute valproate study, a time point of 90 minutes was chosen based on positive results from previous, acute benzodiazepine treatment studies (Owens et al. 1991). Based on the results in Figure 1, injection of 100 mg/kg valproate produces serum values within or near the therapeutic range in humans of 50 to 100 μg/ml (McElroy et al. 1987) for most of the 90-min time period. The mean serum drug concentration at the time of sacrifice in the acute valproate study was 7 ± 2 μg/ml, which is comparable to the predicted value of 9 μg/ml from the regression curve (Figure 1). Serum valproate concentrations in rats treated with 1 or 10 mg/kg were, in contrast, less than 1 μg/ml at the time of sacrifice.

A dose-finding study was next conducted to determine the relationship between continuous valproate dosing and serum concentrations. Rats were treated with 175, 230, 600, or 1,424 mg/kg/day (the solubility limit) of sodium valproate. The 175 mg/kg/day group received the drug in single Alzet 2ML2 minipumps, the 230 and 600 mg/kg/day groups received single 2ML1

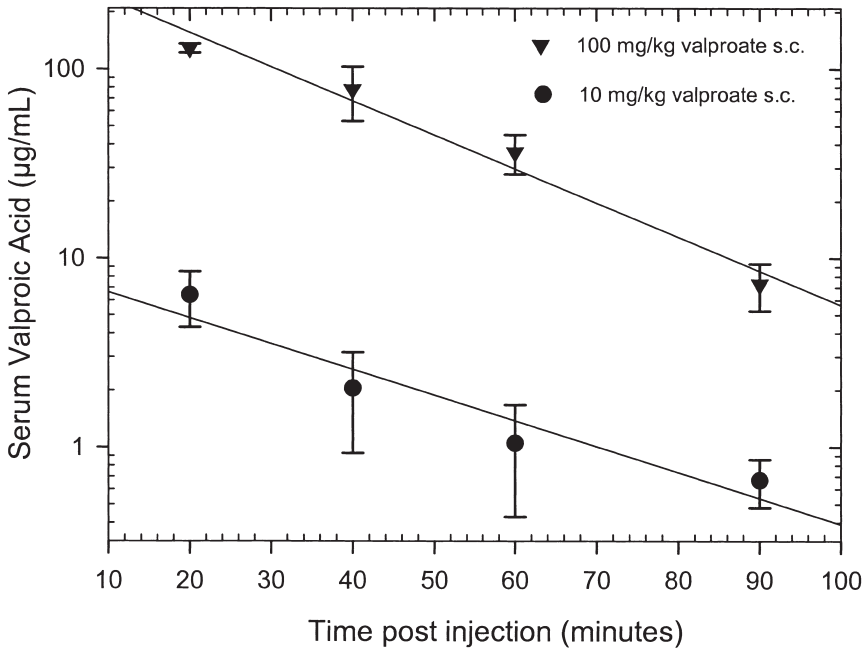

Figure 1. Elimination pharmacokinetics of valproate in rat. Rats were injected SC with 1, 10, or 100 mg sodium valproate and sacrificed at the indicated times. $n = 5$ per group. Serum valproic acid concentrations were <1 μg/ml at all time points in the 1 mg/kg treatment group.

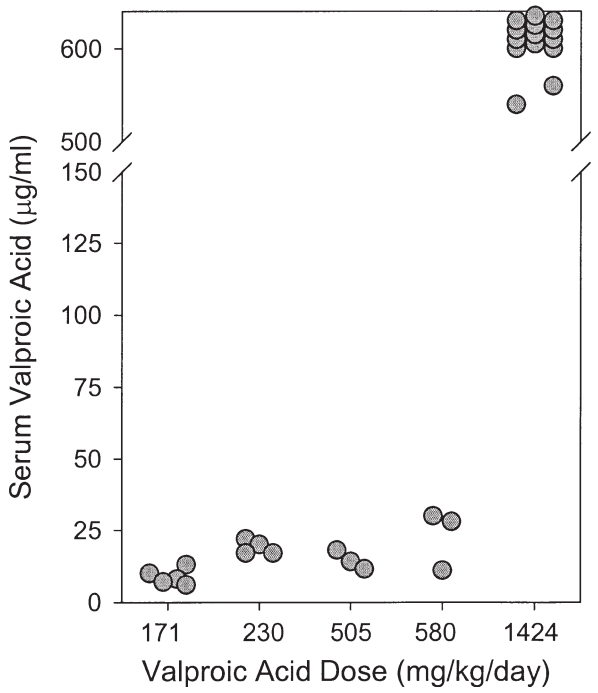

Figure 2. Relationship between chronic valproate dose and serum concentration. Rats were administered sodium valproate for 1 week via osmotic minipump as follows: 175 mg/kg/day (Alzet 2ML2), 230 mg/kg/day (Alzet 2ML2), 600 mg/kg/day (Alzet 2ML2), or 1424 mg/kg/day (two Alzet 2ML2 pumps). The rats in the high-dose group became moribund and were sacrificed after 20 hours.

minipumps, and the 1,424 mg/kg/day group received two model 2ML1 minipumps. The high-dose group became obtunded or died within 24 hours of minipump implantation, and the surviving rats were immediately killed. Rats in the other dose groups were killed after 7 days of continuous drug administration. Assuming steady-state pharmacokinetics (>5 half lives) at the time of death, there was a nonlinear relationship between serum drug concentration and valproate dose (Figure 2), with an abrupt increase between 600 and 1,424 mg/kg/day.

On the basis of these results, an intermediate valproate dose of 875 mg/kg/day was chosen for the subsequent experiment. Treatment of 14 rats with 875 mg/kg/day for 1 week resulted in a mean serum valproate concentration of 38 ± 4 μg/ml (n = 13; range 22–60 μg/ml), which is close to the therapeutic range observed in humans. Animals receiving this dose appeared healthy for the duration of treatment, and their behavior was grossly indistinguishable from that of controls.

Acute Valproate Effects on CRF Concentrations

Subcutaneous injection of 1, 10, or 100 mg/kg sodium valproate did not result in any statistically significant changes in CRF concentrations in 10 brain regions examined, nor were any notable, dose-related trends observed (Table 1).

Chronic Valproate Effects on CRF Concentrations

One-week valproate adminstration resulted in a 21% increase in CRF concentrations in the frontal cortex, a 30% decrease in the median eminence, and a 35% decrease in the midbrain raphe nuclei (Table 2), with no statistically significant changes in the four other brain regions analyzed.

Chronic Valproate Effects on CRF mRNA Expression

One-week valproate administration resulted in a 14% decrease in CRF mRNA expression in the PVN, and an 18% decrease in the CeA (Figure 3, Table 3). There was no statistically significant effect of valproate treatment on CRF mRNA expression in the ventral + dorsolateral BNST. There was also no effect within the dorsolateral subdivision alone.

Table 1. Acute Valproate Effects on CRF Concentration

Brain Region	Vehicle (pg/mg protein)	Valproate 1 mg/kg	Valproate 10 mg/kg	Valproate 100 mg/kg
Prefrontal cortex	21.5 ± 4.4	19.5 ± 2.8	16.2 ± 1.8	25.8 ± 5.6
Frontal cortex	18.4 ± 1.8	18.7 ± 1.9	19.4 ± 1.7	19.5 ± 2.3
Piriform cortex	18.6 ± 1.2	16.5 ± 0.7	16.4 ± 1.0	17.2 ± 0.8
Hypothalamus	27.4 ± 1.3	26.3 ± 1.4	27.1 ± 2.5	27.9 ± 1.4
Median eminence	813 ± 49	908 ± 40	945 ± 27	824 ± 35
Amygdala	30.3 ± 1.4	32.1 ± 1.5	31.5 ± 1.0	28.9 ± 0.8
Hippocampus	11.1 ± 0.9	11.5 ± 0.8	10.3 ± 0.6	12.1 ± 0.9
Midbrain raphe	20.7 ± 2.5	15.1 ± 1.7	16.5 ± 1.3	18.0 ± 2.0
Locus coeruleus	14.8 ± 0.8	15.7 ± 1.1	16.2 ± 1.6	17.0 ± 1.9
Cerebellum	6.56 ± 0.62	7.29 ± 0.77	6.79 ± 0.84	6.88 ± 0.64

Rats were administered vehicle or sodium valproate (1, 10, or 100 mg/kg) and sacrificed 90 minutes later. CRF concentrations were determined in dissected brain regions by radioimmunoassay. n = 10 to 12 per group except 7 to 9 per median eminence group. All data expressed as mean ± SEM.

Table 2. Chronic Valproate Effects on CRF Concentrations

Brain Region	Vehicle	Valproate
Prefrontal cortex	17.8 ± 2.2 pg/mg	13.8 ± 2.0
Frontal cortex	25.0 ± 1.8	30.3 ± 1.6*
Median eminence	2680 ± 160	1880 ± 170**
Hippocampus	15.5 ± 1.5	17.4 ± 1.5
Midbrain raphe nuclei	96.4 ± 9.4	63.1 ± 5.1**
Locus coeruleus	15.6 ± 2.2	11.8 ± 1.1
Cerebellum	2.96 ± 0.32	3.01 ± 0.48

Rats were treated with vehicle or 875 mg/kg/day sodium valproate for 1 week. CRF concentrations were determined in the indicated brain regions by radioimmunoassay (mean ± SEM). $n = 10$ in vehicle group, 14 in valproate group. *$p < .05$; **$p < .01$ (t-test with correction for multiple comparisons).

Table 3. Chronic Valproate Effects on CRF mRNA Expression

Brain Region	Control	Valproate
Paraventricular nucleus	796 ± 31 nCi/mg	683 ± 38*
Bed nuc. stria terminalis	278 ± 10	293 ± 10
dorsolateral subdivision	298 ± 23	350 ± 23
Central nuc. amygdala	263 ± 12	217 ± 9**

Rats were treated with vehicle or 875 mg/kg/day sodium valproate for 1 week. CRF mRNA density was determined by *in situ* hybridization histochemistry (mean ± SEM). $n = 10$ in vehicle group, 13 in valproate group. *$p < .05$; **$p < .01$ (t-test with correction for multiple comparisons).

Chronic Valproate Effects on CRF_1 Receptor mRNA Expression

CRF_1 receptor mRNA density was unchanged in the frontoparietal cortex of rats treated for 1 week with vehicle or valproate. A 10% decrease in CRF_1 receptor mRNA expression in the basolateral amygdala of valproate-treated rats did not reach statistical significance ($p = .11$).

Acute and Chronic Valproate Effects on HPA Axis Activity

Acute administration of sodium valproate did not result in any changes in plasma ACTH or corticosterone concentrations, although there was a slight trend toward a dose-dependent increase in corticosterone values (Table 4). One-week valproate administration did not alter ACTH concentrations at the time of sacrifice, but did increase mean corticosterone concentrations by 3.6-fold (note: acute and chronic treatment groups were assayed separately; interexperiment comparisons are invalid because of potential assay variation).

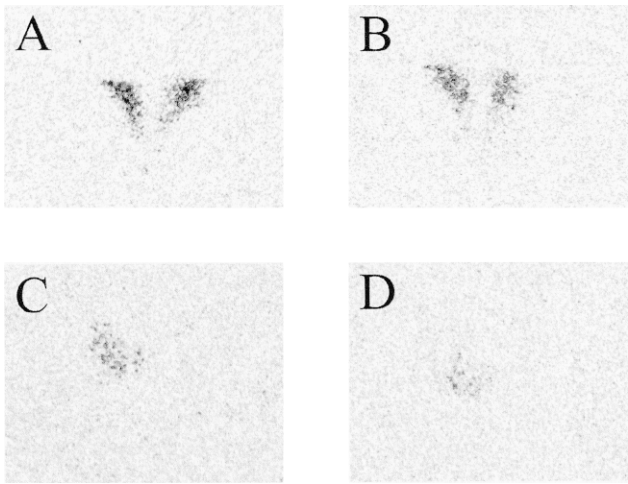

Figure 3. In situ hybridization analysis of CRF mRNA expression in the hypothalamic paraventricular nucleus (PVN) and central nucleus of the amygdala (CeA). Representative images were chosen that were closest to the average mRNA density within each treatment group. (**A**) Control PVN; (**B**) valproate PVN; (**C**) control CeA; (**D**) valproate CeA.

DISCUSSION

Although valproate has been administered to rats in a number of studies (most of which examined GABAergic neurotransmission), there is relatively little published information concerning its pharmacokinetic parameters. In two previous reports, the elimination half life in plasma was reported to be 18 (Heinemeyer et al. 1985) or 60 min (Mesdjian et al. 1982). In our study, the apparent half life in rat serum following SC administration was 17 min. The extremely rapid elimination profile of valproate poses a challenge for single-dose studies, because it is important both to allow sufficient time for neurochemical adaptations to take place and to avoid reversal of the effects attributable to drug elimination. In this study, a 90-min time point was chosen, taking these two factors into consideration.

Acute valproate treatment did not alter CRF concentrations in any brain region examined, nor were HPA axis hormones significantly affected (Table 4). There was a trend toward a dose-related *increase* in plasma corticosterone concentration following valproate administration. The lack of a parallel increase in the major stimulatory hormone, ACTH, would disfavor the interpretation of this increase secondary to an action on the anterior pituitary gland. However, chronic valproate administration also produced an elevation in plasma corticosterone concentrations without any concomitant rise in ACTH concentrations. It is possible that valproate produces a direct effect at the level of the adrenal cortex, or perhaps increases production of a adrenocortical-stimulating hormone other than ACTH.

There are a number of possible explanations for the absence of acute valproate effects on CRF concentra-

Table 4. Plasma ACTH and Corticosterone Concentrations

	Acute, Control	Acute, 1 mg/kg	Acute, 10 mg/kg	Acute, 100 mg/kg	Chronic Control	Chronic Valproate
ACTH (pg/ml)	10.1 ± 2.1	8.8 ± 1.3	5.9 ± 1.1	11.4 ± 2.7	19.8 ± 3.4	15.5 ± 3.2
Corticosterone (ng/ml)	9.4 ± 1.5	8.5 ± 1.0	11.9 ± 3.7	15.2 ± 4.5	17.8 ± 6.3	64.9 ± 15.5*

Plasma ACTH and corticosterone were determined from trunk blood of rats given sc injections of vehicle or 1–100 mg/kg valproate 90 minutes before sacrifice ("acute"), or treated with vehicle or 875 mg/kg/day sodium valproate for 1 week via minipumps ("chronic"). n = 10–14 per group. *$p < .05$, valproate vs. vehicle control.

tions in this study. The 1 and 10 mg/kg doses may well have been pharmacodynamically inactive throughout the dosing interval. The 100 mg/kg dose most likely produced therapeutic serum concentrations during approximately half the treatment duration, but a "rebound" effect of CRF neurons during the last 30 to 45 min as valproate was rapidly removed from circulation cannot be ruled out as a potential confound. Even if drug concentrations were constant, it is possible that alterations in CRF synthesis, storage, or release would produce changes in CRF concentrations either before or after the 90-min time point. Finally, valproate is not known to have an immediate antimanic effect, but typically requires at least a few days of treatment; similarly, chronic drug administration may alter CRF neuropeptide systems via delayed, plastic, neuroregulatory processes.

To address these issues, a second experiment was conducted in which valproate was continuously infused over a 1-week period. A pilot dosing study revealed two technical challenges to performing this study. First, valproate administration was rapidly fatal in rats at a dose of 1424 mg/kg/day that was expected to yield significantly lower serum concentrations than observed based upon the prior experiments (Figure 2). A literature search failed to locate a known, pathologic basis of acute valproate toxicity in rats; a rare incidence of fulminant heptatoxicity in humans has been reported (Eadie et al 1988). A second challenge is that the relationship between dose and serum concentration is nonlinear; an abrupt increase in serum concentration was observed between the highest two doses in the pilot study (Figure 2). This pattern is characteristic of drugs that exhibit saturation of hepatic clearance mechanisms or protein binding. Following saturation, elimination of drugs becomes zero order rather than first order. Saturation of plasma protein binding can lead to excess free drug that may lead to toxicity; however, this does not explain the huge increase in total serum valproic acid concentrations. These challenges notwithstanding, a chronic dose of valproate was selected that produced a mean serum drug concentration (38 ± 4 μg/ml) near the low end of therapeutic concentrations obtained in humans. Higher steady-state concentrations than those obtained in the present study may be difficult to attain without further studies examining dose–plasma concentration relationships above 875 mg/kg/day.

Chronic valproate administration resulted in a ⩾30% decrease in CRF concentrations in the median eminence and raphe nuclei, and a 21% *increase* in the frontal, but not prefrontal, cortex. Smaller decreases, also statistically significant, in CRF mRNA expression were observed in the PVN and CeA. The increase in CRF concentration within the frontal cortex that was observed after chronic valproate treatment is difficult to explain conceptually. The parallel decreases in PVN CRF mRNA and median eminence CRF concentrations likely reflect a single effect of valproate on CRF synthesis within the neuroendocrine pathway. Alternatively, decreases in CRF concentration in the median eminence could be explained by increased CRF secretory activity and nerve terminal peptide depletion. That plasma ACTH concentrations were not elevated in this study disfavors but does not entirely rule out this alternative explanation. For the statistically significant findings in other brain regions, there was no opportunity to compare mRNA and peptide effects within the same pathway. The decreases in CeA CRF mRNA and raphe CRF concentration would be consistent with reduced CRF synthesis and transport along the CeA-raphe pathway (Gray 1993), but these sites may contain many other CRF efferent and afferent projections, respectively.

Despite the valproate-induced decreases in PVN CRF mRNA and median eminence CRF concentration, there was no evidence of reduced HPA axis activity. In fact, similar to the trend observed after acute drug treatment, plasma corticosterone concentration was substantially *increased* without a concomitant increase in ACTH levels. The mechanism(s) underlying this effect is unclear. It is possible that this increase in corticosterone concentration was responsible for some of the observed changes in CRF mRNA expression and peptide concentration. Decreases and increases in corticosterone concentration, produced either by adrenalectomy or by exogenous steroid administration, respectively, are known to cause reciprocal alterations in PVN CRF mRNA expression (Jingami et al. 1985; Young et al. 1986; Kovács and Makara 1988; Makino et al. 1994a). However, corticosterone has been shown to regulate CRF mRNA expression in the CeA in an oppo-

site manner to regulation in the PVN (Palkovits et al. 1998; Makino et al 1994a, 1994b); whereas, parallel decreases in these two nuclei occurred in the present study. Therefore, it is likely that valproate produced at least some of the CRF mRNA and peptide changes through neuronal effects independent of corticosterone feedback.

Benzodiazepines are anxiolytic and sedative drugs that share with valproate a positive action on GABAergic neurotransmission. Benzodiazepines also decrease CRF mRNA expression in the PVN and CeA (Imaki and Vale 1993; Skelton et al. 2000). The effects of valproate treatment on CRF concentrations in rat brain were different in this study than the acute and chronic alprazolam effects previously reported (Owens et al. 1991). Whereas the strongest findings in the current study were in the raphe nuclei and median eminence, the benzodiazepine results were most notable for decreased CRF concentration in the locus ceruleus. Another difference in the present results is that CRF_1 receptor mRNA expression was not affected by chronic valproate treatment in the frontoparietal cortex or basolateral nucleus of the amygdala; whereas, benzodiazepine administration decreases both CRF_1 receptor mRNA expression and binding in these areas (Skelton et al. 2000). Further investigation will be required to elucidate the mechanisms governing differential regulation of CRF concentration in various brain regions by the two classes of psychopharmacologic agents.

In addition to being an efficacious antimanic compound, valproate may also exert antidepressant effects in bipolar patients when administered chronically (reviewed, Keck and McElroy 1998). One potential shared mechanism of action of valproate and several antidepressants (e.g., venlafaxine, paroxetine, reboxetine) is its reduction in CRF mRNA expression. In addition, valproate is well known for its anticonvulsant properties. Although we hypothesized that valproate administration may alter CRF neuronal function as part of its mechanism of action as a mood-stabilizing agent, the possibility that such an effect is involved in seizure protection cannot be discounted. Indeed, CRF possesses potent proconvulsant activity, particularly in neonatal rats (Baram and Schultz 1991; Weiss et al. 1992).

In summary, the present study indicates that subchronic administration of sodium valproate causes a reduction in CRF mRNA expression in the PVN and CRF concentration in its primary projection field, the median eminence. CRF mRNA expression in the CeA and CRF concentration in the raphe nucleus were also decreased. Neither of the effects on regional CRF concentrations occurred after acute valproate treatment at the doses and time point examined. The fact that decreased CRF concentrations were observed in the raphe nucleus is of particularly interest. It was recently demonstrated that CRF has predominately inhibitory actions on the firing rate of serotonergic neurons in the dorsal raphe and 5-HT release in the striatal terminal field (Price et al. 1998). Thus, decreased CRF release within this region would be expected to affect serotonergic neurotransmission positively. Indeed, as noted above, serotonin transporter antagonists, which increase serotonergic neurotransmission by a different mechanism, are also particularly effective agents for the depressive phase of bipolar illness (Kalin 1997). The connection between serotonin systems and bipolar depression is further supported by the genetic association between such serotonin-related genes as the tryptophan hydroxylase gene and the bipolar phenotype (Bellivier et al. 1998; Vincent et al. 1999). The present relationship between valproate treatment and CRF neuronal activity in discrete brain regions may provide the impetus for further study of CRF alterations in bipolar disorder by postmortem examination or functional brain-imaging methods that may yield greater sensitivity than CSF peptide concentrations.

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