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Valproate improves prepulse inhibition deficits induced by corticotropin-releasing factor independent of $GABA_A$ and $GABA_B$ receptor activation

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ABSTRACT

Corticotropin-releasing factor (CRF) is implicated in the pathogenesis of bipolar disorder, an illness associated with deficits in prepulse inhibition (PPI) of the acoustic startle response. Valproate is used in the treatment of bipolar disorder and may alter CRF activity via a $GABA_A$-ergic mechanism. This study determined the effect of valproate on CRF-disrupted PPI and examined the role of the hypothalamic–pituitary–adrenal axis and GABA-ergic signaling in the effect of valproate. Valproate (60–240 mg/kg) dose-dependently reversed PPI deficits displayed by transgenic mice overexpressing CRF (CRFtg), and normalized PPI deficits induced by CRF i.c.v. infusion in 129Sv mice. Valproate enhanced corticosterone secretion more effectively in CRFtg than in wild-type mice. The effect of valproate on PPI was not blocked by the $GABA_A$ receptor antagonist bicuculline, the $GABA_B$ receptor antagonists phaclofen and SCH 50911 or combined administration of a $GABA_A$ and $GABA_B$ receptor antagonist. The beneficial effect of valproate on PPI was not mimicked by the $GABA_A$ receptor agonist muscimol, the GABA transaminase inhibitor vigabatrin, the histone deacetylase (HDAC) inhibitor sodium butyrate or by the mood stabilizers lithium, carbamazepine, lamotrigine or topiramate.

Thus, we showed that valproate improves CRF-induced PPI deficits, albeit via a so far unknown mechanism. These marked beneficial effects of valproate on CRF-induced sensorimotor gating deficits suggest that valproate may be of particular value in specific subgroups of bipolar patients that are characterized by alterations in the CRF system.

1. Introduction

Cumulative evidence indicates that stress is an important factor in the pathogenesis of schizophrenia and bipolar disorder (Britzke et al., 2012). The corticotropin-releasing factor (CRF) system takes a cardinal position in the regulation of stress responses (Lloyd and Nemeroff, 2011). Decreased levels of CRF-binding protein mRNA were found in amygdala post-mortem tissue from male schizophrenic subjects (Herringa et al., 2006), and positive treatment response to the antipsychotic quetiapine was associated with a decrease in CRF levels in CSF of schizophrenia patients (Nikisch et al., 2011). Furthermore, genetic variations in CRF-related genes have been associated with schizophrenia, schizoaffective disorder and bipolar disorder (reviewed by Binder and Nemeroff (2010)).

These disorders are associated with deficits in prepulse inhibition (PPI) of the acoustic startle response (Braff et al., 1978); that is, the reduction in the startle reflex produced by a weak prepulse stimulus. In schizophrenia patients, deficient PPI is thought to reflect a loss of sensorimotor gating that may lead to sensory flooding and the inability to filter irrelevant thoughts from intruding into awareness (Braff et al., 1978, 2008). In rodents, both intraventricular (i.c.v.) infusion of CRF and long-term central CRF overexpression disrupt PPI (Conti et al., 2005; Dirks et al., 2002, 2003; Risbrough et al., 2003). Interestingly, transgenic mice overexpressing central CRF (CRFtg) show neuroendocrine changes (Groenink et al., 2002) that are reminiscent of bipolar disorder (Langan and McDonald, 2009) and schizophrenia (Walker et al., 2008). Moreover, the disrupted PPI response of CRFtg mice is

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improved by antipsychotics such as haloperidol, risperidone, and clozapine (Dirks et al., 2003).

Valproate is a mood stabilizer that has been used for the treatment of bipolar disorder (Bowden and Singh, 2005) and, to a lesser extent, schizophrenia (Schwarz et al., 2008). Definitive mechanisms mediating its clinical efficacy are unclear, but enhancement of GABAergic neurotransmission through inhibition of the metabolic enzyme GABA transaminase (Johannessen, 2000), reduction of neuronal excitability by affecting intracellular signaling pathways (Landmark, 2007) and inhibition of histone deacetylase (HDAC) (Lee et al., 2012) may play a role. In rats, valproate has been shown to alter the expression of CRF and its receptors (Gilmor et al., 2003; Stout et al., 2001). In addition, valproate inhibited CRF secretion and synthesis in rat hypothalamic neurons *in vitro*. These effects could be blocked by the $GABA_A$ receptor antagonist bicuculline and be mimicked by the $GABA_A$ receptor agonist muscimol, suggesting involvement of increased $GABA_A$ receptor activation (Tringali et al., 2004). These are interesting findings, given the putative role of CRF in the pathophysiology of bipolar disorder and schizophrenia, and the proposed alterations in the GABA-ergic system upon prolonged exposure to elevated CRF levels (Bagosi et al., 2008; Cook, 2004; Kirby et al., 2008; Rainnie et al., 2004; Vinkers et al., 2012). The effects of valproate on CRF-induced behavioral alterations however, have not been studied so far. Such studies could contribute to our knowledge of mechanisms relevant for the etiology and treatment of these stress-related disorders.

Here we show that compared to other commonly used mood stabilizers, valproate is particularly effective in improving CRF-induced PPI deficits. Next we studied several potential mechanisms underlying the beneficial effect of valproate on PPI in CRFtg mice, including GABAergic signaling, the HPA axis and inhibition of histone deacetylase (HDAC).

2. Materials and methods

2.1. Animals

The experiments were performed according to the Guide for Care and Use of Laboratory Animals and were approved by the Ethical Committee for Animal Research of Utrecht University. All efforts were made to minimize animal discomfort, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

CRFtg mice (line 2122, eighteenth generation) were generated as previously described (Dirks et al., 2002). Briefly, the CRF transgene was composed of the complete coding sequence of rat CRF cDNA (600 bp fragment), which was inserted into a 8.2-kb genomic DNA-fragment encompassing the murine Thy-1.2 gene, including regulatory regions and polyadenylation signal sequence. Thy-1 regulatory sequences drive constitutive transgene expression in postnatal and adult neurons. Subsequent breeding at the local breeding facilities (Utrecht, the Netherlands) consisted of matings between heterozygous transgenic males (C57BL/6J background) and C57BL/6JIco females (Charles River, the Netherlands). Genotyping was done by PCR. For PPI and corticosterone experiments, male CRFtg mice, 9–16 weeks old, were used, with their wild-type (WT) littermates serving as control mice.

The CRF infusion PPI experiment was performed on male 129SvEvTac mice, 10–16 weeks old (Taconic, Denmark). This strain was chosen, because it is more sensitive to the effects of CRF on PPI (Risbrough et al., 2003).

Animals were group-housed in bedded plastic cages (Makrolon type 2L), enriched with a piece of PVC-tubing and paper tissue, at constant room temperature (21 ± 2 °C) and relative humidity (50–60%). Mice were maintained on a 12:12 light/dark cycle (lights on: 06:00–18:00 h). Standard rodent food pellets (Special Diet Services, Witham, Essex, United Kingdom) and water were freely available.

2.2. CRF infusion: surgery

One week after arrival, the animals were systemically anesthetized using isoflurane gas anesthetic (2–3%, Isoflo, Abbott) mixed with oxygen and nitrous oxide. In the wound space, an additional local anesthetic was applied (Lidocaïne 5%, Alfacaine, Alfasan). Each mouse was prepared with a 23 gauge 2.5-mm-length unilateral guide cannula (Plastics One) into the lateral ventricle (flat skull; anteroposterior, −0.2 mm; mediolateral, +1 mm; dorsoventral, −2.5 mm from bregma), which was secured with dental cement. To make sure the cement would be held in place, shallow lines were carved into the skull. Plastic dummies were used to close the cannulae. Before onset of testing, the animals were allowed to recover for one week. At the end of the experiment, cannula placements were assessed by infusion of methylene blue dye and verification of dye in the ventricular system (no mice excluded).

2.3. Corticosterone determination

Mice were randomly allocated to a treatment group and decapitated 30 min after valproate treatment (0, 120, 240 mg/kg IP). Blood samples were collected between 9:00 AM and 12:30 PM. Trunk blood (500–700 μl) was collected in ice-cooled Eppendorf cups containing 25 μl (0.21 M) ethylenediamine tetra-acetate. Plasma was separated by centrifugation (3000 rpm for 10 min at 4 °C), and aliquots were stored at −70 °C until assayed. Plasma corticosterone concentrations were measured in duplicate using a double antibody radioimmunoassay for rat corticosterone (ICN Biochemicals, Zoetermeer, the Netherlands).

2.4. Drugs

Valproic acid sodium salt (a.k.a. valproate) (60, 120, 240 mg/kg), bicuculline methiodide (2, 5 mg/kg), phaclofen (3-Amino-2-(4-chlorophenyl)propanephosphonic acid) (7.5, 15 mg/kg), SCH 50911 ((2*S*)-(+)-5,5-Dimethyl-2-morpholineacetic acid) (15, 30 and 60 mg/kg), muscimol (0.33, 1.0, 3.0 mg/kg) (3-Hydroxy-5-aminomethyl-isoxazole, 5-Aminomethyl-3-hydroxy-isoxazole, 5-Aminomethyl-3-isoxazolol), vigabatrin ((±)-γ-Vinyl-GABA) (200, 400, 800 mg/kg), sodium butyrate (0.3, 0.6, 1.2 mg/kg), and lithium chloride (10, 20, 40 mg/kg), were dissolved in saline. Carbamazepine (5*H*-Dibenz[*b*,*f*]azepine-5-carboxamide) (20, 40, 80 mg/kg), lamotrigine (6-(2,3-Dichlorophenyl)-1,2,4-triazine-3,5-diamine, GI 267119X) (3, 9, 27 mg/kg) and topiramate (2,3:4,5-*Bis*-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate) (30, 100, 300 mg/kg) were suspended in a vehicle, containing saline and Tween 80 (3%). Solutions were freshly prepared daily. Valproate, bicuculline, phaclofen, vigabatrin, lamotrigine and sodium butyrate were obtained from Sigma–Aldrich (Steinheim Germany); the other drugs were gifts from Servier, Croissy/Seine France. Compounds were administered intraperitoneally (IP) in a volume of 10 ml/kg, 30 min before the start of the test, except for vigabatrin which was injected 5 h before the test. Drug doses were chosen based on literature reporting behavioral effects (muscimol and bicuculline Egashira et al., 2013; SCH 50911 Colombo et al., 2001; phaclofen Escher and Mittleman, 2004; vigabatrin Griffin et al., 2012; sodium butyrate Schroeder et al., 2007), and dose–response pilots (bicuculline and phaclofen, not shown). Dose ranges, route of administration (IP) and injection test interval (30 min) in which valproate, lithium, carbamazepine, lamotrigine and topiramate