# $CRF_1$ Not Glucocorticoid Receptors Mediate Prepulse Inhibition Deficits in Mice Overexpressing CRF

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**Background:** Both corticotropin-releasing factor (CRF) and glucocorticoid receptors (GR) are implicated in the psychotic symptoms of psychiatric disorders. Correspondingly, it is of interest to determine their respective involvement in the sensorimotor gating deficits displayed by transgenic mice overexpressing CRF. These mice reveal lifelong elevations of CRF and corticosterone levels.

**Methods:** Effects of the GR antagonists ORG34517 (5–45 mg/kg by mouth [PO]) and mifepristone (5–45 mg/kg PO) and the $CRF_1$ receptor antagonists CP154,526 (20–80 mg/kg intraperitoneally [IP]) and DMP695 (2.5–40.0 mg/kg IP) on prepulse inhibition (PPI) of the acoustic startle response were studied in mice overexpressing CRF and in their wild-type littermates. In addition, PPI was measured in both genotypes 2 weeks after adrenalectomy with or without exogenous corticosterone administration via subcutaneous pellet implant (20 mg corticosterone).

**Results:** ORG34517 and mifepristone did not influence perturbation of PPI in mice overexpressing CRF; reducing corticosterone levels by adrenalectomy likewise did not improve PPI. Further, elevation in corticosterone levels by pellet implantation did not disrupt PPI in wild-type mice. Conversely, both $CRF_1$ receptor antagonists, CP154,526 (40–80 mg/kg IP) and DMP695 (40 mg/kg IP), significantly restored PPI in CRF-overexpressing mice.

**Conclusions:** Sustained overactivation of $CRF_1$ receptors rather than excessive GR receptor stimulation underlies impaired sensorimotor gating in CRF-overexpressing mice. $CRF_1$ receptors thus may play a role in the expression of psychotic features in stress-related psychiatric disorders.

**Key Words:** Corticosterone, CRF (corticotropin-releasing factor), CRH (corticotropin-releasing hormone), major depression, psychosis, sensorimotor gating

Altered hypothalamic–pituitary–adrenal (HPA) axis function is observed in several psychiatric disorders but is especially pronounced in major depression with psychotic features (1–3). Patients with psychotic depression consistently show a high rate of dexamethasone nonsuppression, markedly higher levels of 24-hour urinary free cortisol (4,5), and elevated cortisol during evening hours (6). Therefore, a causal relationship between increased cortisol secretion and clinical symptoms (1–3,7) has been suggested. It has been hypothesized that psychotic symptoms of psychotic depression reflect increased mesolimbic dopaminergic activity secondary to HPA axis overactivity and, accordingly, that glucocorticoid receptor (GR) antagonists, which block cortisol effects, might be effective in the treatment of this disorder (7–11). Recently, clinical studies appeared suggesting that GR antagonists alleviate psychotic symptoms in patients suffering from major depression with psychotic features (10,12–14), although this has been disputed (15). Indeed, despite elevated activity of the HPA axis and higher cortisol levels, other mechanisms may be implicated.

A potentially important candidate is the neuropeptide, corticotropin-releasing factor (CRF), which not only activates the HPA axis in response to stress, but also modulates behavioral responses to a wide range of stressors (2–3,16–20). During stress, CRF is released from the paraventricular nucleus of the hypothalamus and, via $CRF_1$ receptor activation, enhances corticotropin (ACTH) release from the pituitary that subsequently enhances cortisol secretion (corticosterone in rodents) from the adrenal cortex (1,2). In parallel, CRF brain circuits located outside the hypothalamus mediate emotional responses and arousal (3,16–18,21) via $CRF_1$ and $CRF_{2\alpha}$ receptors (22–24). In depressive disorders, alterations in both the neuroendocrine and extra-hypothalamic CRF system have been observed (25). Moreover, $CRF_1$ receptor antagonists show antidepressant properties in rodents and preliminary studies indicate that depression scores may be reduced following $CRF_1$ receptor blockade in man (3,16,17,26).

Several studies indicate that CRF hypersecretion may also be related to psychotic symptoms. Thus, a decrease in CRF-binding protein was seen in the amygdala of male schizophrenia patients (27), and $CRF_1$ receptors are implicated in stress-triggered psychostimulant "seeking" and activation of mesolimbic dopaminergic projections, processes related to onset of psychosis (8,11,20,28,29). Interestingly, schizophrenia is associated with perturbed activity of the HPA axis, and the antipsychotic quetiapine suppressed stress-induced induction of CRF in the hypothalamus (30,31). Furthermore, patients suffering from posttraumatic stress disorder (PTSD) with secondary psychotic features have significantly higher CSF concentrations of CRF than non-psychotic PTSD and control subjects (32).

A phenomenon commonly associated with disorders exhibiting psychotic features is reduced prepulse inhibition (PPI). PPI is measured as the inhibition of the acoustic startle response, which occurs when a nonstartling stimulus is presented 30–300 msec before the startling stimulus (33). In both humans and animals, PPI is used as an operational measure for sensorimotor gating, a

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neural filtering process that allows attention to be focused on the most salient aspects of the environment (34,35). Prepulse inhibition is reduced in several neuropsychiatric disorders such as schizophrenia, schizotypal personality disorder, obsessive–compulsive disorder, and Huntington's disease (36). To the best of our knowledge, however, PPI has never been measured in patients with psychotic depression.

In animals, long-term CRF overexpression, as well as acute infusion of CRF into the central nervous system (CNS), induces deficits in PPI of the acoustic startle response (37–40). Deficits in PPI induced by CRF infusion in mice are mediated by activation of $CRF_1$ receptors (39), which are located in regions relevant for modulation of PPI, including cortex, hippocampus, striatum, basolateral amygdala, and nucleus reticularis pontis caudalis (23,35). Activation of $CRF_1$ receptors may also affect PPI indirectly by altering the release of dopamine and serotonin (8,41,42), which modulate PPI (34). Finally, CRF exerts its effect on the pituitary through $CRF_1$ receptors and could reduce PPI by enhancing corticosterone secretion. A few animal studies have reported on corticosterone-induced PPI deficits (43–45). As with CRF, corticosterone is in a position to alter PPI directly and indirectly. In the CNS, corticosterone exerts its effect through high-affinity mineralocorticoid receptors (MR) and lower-affinity GR receptors. The MRs are occupied under resting conditions, whereas the GRs are only occupied when corticosteroid levels increase under stress conditions or at the peak of the corticosteron circadian rhythm (1,2). The MRs are mainly expressed in hippocampus, whereas GRs are more widely expressed, including the hypothalamus and areas that regulate PPI including prefrontal cortex, basolateral amygdala, and hippocampus (23,35,46,47). Furthermore, corticosterone may modulate PPI indirectly by altering dopaminergic or serotonergic neurotransmission (3,48–50).

To study the potential involvement of CRF and corticosterone in psychotic features, we used a mouse model of central CRF overexpression. In these transgenic mice, overexpression of CRF begins 4 days after birth and is restricted to the CNS (37). Function of the HPA axis is also dysregulated. Alterations are reminiscent of human depression and include elevated resting corticosterone levels, adrenal hypertrophy, and dexamethasone nonsuppression (2,5,51,52). Furthermore, PPI is disrupted (37), and interestingly, antipsychotics such as haloperidol, risperidone, and clozapine restore PPI deficits in CRF-overexpressing mice (53). The aim of this study was to determine whether the PPI deficits are a direct result of central CRF excess, secondary to elevated corticosterone levels, or both. We first studied the acute actions of the GR receptor antagonists ORG34517 and mifepristone on PPI in mice overexpressing CRF and in wild-type (WT) mice. A second study characterized the significance of elevated levels of corticosterone compared with CRF. We measured PPI in CRF-overexpressing mice subjected to adrenalectomy (ADX) to suppress corticosterone (but not CRF) levels, and in WT mice with high corticosterone levels following pellet implantation. In a third experiment, to determine the involvement of $CRF_1$ receptors in PPI deficits, we evaluated the actions of the $CRF_1$ receptor antagonists CP154,526 and DMP695 in CRF-overexpressing and WT mice.

## Methods and Materials

### Animals

Transgenic mice overexpressing neural CRF were generated as described previously (54). Briefly, the CRF transgene was composed of the complete coding sequence of rat CRF cDNA (.6-kb 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. The 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).

Male transgenic CRF-overexpressing mice (line 2122, fifteenth generation) were used in these experiments. Littermate WT mice served as control mice. Animals were group housed at constant room temperature (21 ± 2°C) and relative humidity (50%–60%), with EnviroDri (BMI, Helmond, the Netherlands) and PVC tubing as cage enrichment. Standard rodent food pellets (Special Diet Services, Witham, Essex, United Kingdom) and water were freely available. Mice were maintained on a 12-hour light–dark cycle (lights on at 6 AM). All experiments were performed in accordance with the governmental guidelines for care and use of laboratory animals and approved by the Ethical Committee for Animal research of the Faculties of Pharmaceutical Sciences, Chemistry and Biology at Utrecht University.

### Drugs

ORG34517 (5, 15, 45 mg/kg) and mifepristone (also known as RU486; 5, 15, 45 mg/kg) (gifts from NV Organon, Oss, the Netherlands) were dissolved in gelatin-mannitol. Drugs were administered orally (PO). CP154,526 (20, 40, 80 mg/kg) and DMP695 (2.5, 10.0, and 40.0 mg/kg) (gifts from Servier, Croissy/Seine France) were administered intraperitoneally (IP) as a suspension in water with a few drops of Tween80. All drugs were administered 30 min before testing in a volume of 10 mL/kg. Drug structures were as follows: ORG34517 (11ß,17ß)-11-(1,3-benzodioxol-5-yl)-17-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one; mifepristone 17ß-hydroxy-11ß-(4-dimethylaminophenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one); CP154,526 (butyl-ethyl-(2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo(2.3-d)pyrimidin-4-yl)amine)-HCl; DMP695 (N-(2-chloro-4,6-dimethylphenyl)-1-(1-methoxymethyl-(2-methoxyethyl)-6-methyl-1H-1,2,3,triazolo(4,5-c)pyridin-4-amine)-mesylate.

### Surgical Adrenalectomy

Animals 14–16 weeks of age were anaesthetized with an isoflurane, $N_2O/O_2$ mixture. A dorsal incision was made in the skin and lateral incisions in the muscle wall to access the adrenals. Except for the sham-operated, adrenal intact mice, all mice had their adrenals removed. Immediately following ADX, mice were implanted subcutaneously with a solid corticosterone or cholesterol pellet (100 mg, 9 × 2 mm). Corticosterone pellets consisted of 20% corticosterone (ICN Biomedicals, Aurora, Ohio) in cholesterol vehicle (cholesterol 95% stabilized, Acros Organics, Geel, Belgium).

### Startle Apparatus and Experimental Procedures

Startle reflexes were measured in four identical startle response systems (SR-LAB, San Diego Instruments, San Diego, California) consisting of a nonrestrictive Plexiglas cylinder (inner diameter 4 cm, length 13 cm) and grid floor, mounted on a Plexiglas platform and placed in a ventilated, sound-attenuated chamber. Cylinder movements were detected and measured by a piezoelectric element mounted under each cylinder. A dynamic calibration system (San Diego Instruments) was used to ensure comparable startle magnitudes across the four devices. Throughout the session, the startle system delivered a constant back-