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Increased antidepressant sensitivity after prefrontal cortex glucocorticoid receptor gene deletion in mice

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Abstract

Our laboratory has previously shown that antidepressants regulate glucocorticoid receptor (GR) expression in the prefrontal cortex (PFC). To determine if PFC GR are involved in antidepressant effects on behavior or hypothalamic-pituitary-adrenocortical (HPA) axis activity, we treated floxed GR male mice with saline or 15 or 30 mg/kg/d imipramine after PFC injection of adeno-associated virus 2/9 vectors transducing expression of Cre recombinase, to knockdown GR (PFC-GRKD), or green fluorescent protein (PFC-GFP), to serve as a control. The pattern of virally-transduced GR deletion, common to all imipramine treatment groups, included the infralimbic, prelimbic, and medial anterior cingulate cortex at its largest extent, but was confined to the prelimbic and anterior cingulate cortex at its smallest extent. PFC GR knockdown increased behavioral sensitivity to imipramine, with imipramine-treated PFC-GRKD but not PFC-GFP mice exhibiting significant decreases in depression-like immobility during forced swim. PFC GR deletion did not alter general locomotor activity. The 30 mg/kg dose of imipramine increased plasma corticosterone levels immediately after a 5-min forced swim, but PFC GR knock-down had no significant effect on plasma corticosterone under these experimental conditions. We conclude that PFC GR knock-down, likely limited to the medial prelimbic and anterior cingulate cortices, can increase behavioral sensitivity to antidepressants. These findings indicate a novel role for PFC GR in influencing antidepressant response.

Keywords

prefrontal cortex; glucocorticoid receptor; forced swim; corticosterone; imipramine; stress

1. Introduction

The prefrontal cortex (PFC) has been linked with the behavioral and endocrine disturbances observed in depression. Activity of Brodmann area 25, the subgenual cingulate division of the prefrontal cortex, is elevated in depressed compared to healthy subjects, decreases in

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patients that respond to antidepressant or behavioral therapy, and has been manipulated by deep brain stimulation to achieve remission in treatment-resistant depression [1, 2]. Activity of the peri- or pregenual anterior cingulate division of the prefrontal cortex (corresponding to Brodman areas 24 and 32) has also been found to predict treatment response in depression [1]. In animals, the prefrontal cortex also exhibits many of the neurochemical and molecular changes associated with therapeutically-relevant behavioral effects of antidepressants [3].

Hypothalamic-pituitary-adrenal (HPA) axis activity is often elevated in depression [4], and the PFC is implicated in this phenomenon as a site for glucocorticoid endocrine feedback. The PFC expresses glucocorticoid receptors (GR) and mineralocorticoid receptors [5–7], and has been shown to mediate glucocorticoid inhibition of HPA activity [8–11]. Decreased GR expression has been reported in the PFC in depression [12] and could contribute to the glucocorticoid feedback resistance thought to be responsible for elevated HPA activity in this disease [4].

Glucocorticoids have a variety of effects in the PFC that are relevant to depression. Glucocorticoids can act within the PFC to impair working memory but enhance consolidation of emotionally-charged memory [13], effects that have respectively been proposed to contribute to the cognitive deficits and anxiety that can occur with depression [14]. Impaired emotional and cognitive functioning in depression has also been linked with glucocorticoid actions to decrease the number and spine density of PFC neurons [5, 15]. Recently, GR knock-down within the infralimbic PFC has also been found to increase depression-like behavior [11], suggesting that GR loss in depression could contribute to psychiatric symptoms as well as aberrant HPA activity in this disease.

Our laboratory has shown that PFC GR are regulated by antidepressants [6, 7]. These findings suggest that antidepressants could achieve some of their therapeutic effects by regulating PFC GR expression. To determine if PFC GR influence antidepressant action, we have assessed imipramine effects on depression-like immobility and plasma corticosterone in floxed GR mice with virally-transduced PFC GR gene deletion.

2. Materials and Methods

2.1 Animals

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Albany Medical College and were consistent with the standards of the National Institutes of Health [16]. Male mice with a floxed GR exon 2 on a pure C57BL/6 background (B6.129S6-*Nr3c1*^{tm2.1Ljm/J}); [17]) were used for all experiments. Mice were housed on a 12:12 light cycle (lights-on, 6:30 am) with ad libitum access to food and water.

2.1.1. Stereotaxic Surgery—Floxed GR mice were 6–9 weeks old at surgery. Mice were anesthetized with 125 mg/kg ketamine, 12.5 mg/kg xylazine and injected bilaterally in the prefrontal cortex with recombinant adeno-associated virus pseudotype 2/9 vectors (see below) designed to transduce expression of either Cre recombinase, to knock down GR (PFC-GRKD), or green fluorescent protein (PFC-GFP), to serve as a control. Virus (1.45×10^{10} genomic copies in 1 μ l phosphate-buffered saline) was injected bilaterally at A/P 2.0

mm, M/L 0.3 mm, D/V –2.4 mm from bregma with a pulled glass micropipette (World Precision Instruments, Sarasota, FL) attached to a pressure microinjector (Microdata Instruments, South Plainfield, NJ). Post-surgical analgesia was provided by 1 mg/kg indomethacin ip (Alexis Biochemicals, San Diego, CA, USA) in 10ml/kg of sterile-filtered 0.1 M sodium carbonate, with 0.001 M indomethacin also available as a separate drinking fluid [18]. Mice were housed singly after surgery for the duration of the experiment.

After a 3 week recovery, mice were injected with saline or 15 or 30 mg/kg imipramine (Sigma, St. Louis, MO) ip once per day for 6 weeks. Daily injections were given after any behavioral tests or manipulations, so that behavioral tests occurred 16–24 h after the previous day's injection. Behavioral tests and blood sampling were conducted at the end of the sixth week of treatment in the order described below and diagrammed in Figure 1. All tests were completed within 3 h of lights-on.

2.1.2. Open Field Test—The open field test was performed in the morning on day 40 of imipramine or saline treatment. Mice were placed in a 42cm × 35cm × 13.5cm basin for 5 min. Total distance traveled was measured automatically from videotapes using Ethovision 8.1 software (Noldus Information Technology, Leesburg, VA).

2.1.3. Forced Swim Test—One day after open field testing, mice underwent a 15-minute swim followed 24 h later by a 5-min test swim. Swims were conducted in 1000 ml beakers filled with 25±1 °C water. This paradigm has been shown to be sensitive to glucocorticoids [19, 20] and is otherwise equivalent to a single forced swim test [21]. Forced swim behavior was videotaped using Ethovision 8.1 software (Noldus Information Technology). Immobility was scored manually from videotapes by a trained observer blind to experimental groups as the time the mouse was floating or making only sporadic movements to keep its nose out of the water. One mouse in the PFC-GRKD, 30 mg/kg imipramine group died in between open field and forced swim testing; therefore, group sizes differ slightly between the open field and forced swim endpoints.

2.2. Virus stocks

Recombinant adeno-associated virus (AAV) pseudotype vectors containing the AAV2 genome packaged in the AAV9 capsid were used to transduce expression of either Cre recombinase (AAV2/9-Cre) or enhanced green fluorescent protein (AAV2/9-GFP). Viruses were packaged and provided by the Gene Therapy Center and Vector Core of the University of Massachusetts Medical School, Worcester, MA [22]. The respective genes were under the control of a promoter containing the cytomegalovirus immediate-early (CMV IE) enhancer and 260 bp (nucleotides –1261 to –1,001) of the chicken β -actin 6 promoter (personal communication, Li Zhong, University of Massachusetts Medical School Gene Therapy Center and Vector Core).

2.3. Plasma corticosterone assay

Blood was collected by submandibular venipuncture with a Goldenrod 4 mm lancet (Medipoint, Mineola, NY) immediately after the 5 min test swim. Plasma was separated by

centrifugation and assayed for corticosterone using a kit from MPBiomedical (Solon, OH) as previously described [23].

2.4. Immunocytochemistry

Mice were injected with 10 mg/kg dexamethasone to maximize GR nuclear translocation for immunocytochemical detection. Mice were anesthetized 1 h after dexamethasone injection with a lethal dose of sodium pentobarbital (100 mg/kg, ip) and perfused intracardially with normal saline followed by 4% paraformaldehyde in phosphate-buffered saline. Brains were post-fixed 24 h in 4% paraformaldehyde, cryoprotected in 30% sucrose, and sectioned at 25 μ m. Sections were stored in cryoprotectant (50% 0.05M Tris-buffered saline, pH 7.5, 30% ethylene glycol, 20% glycerol) at -20°C .

GR immunoreactivity was detected with a rabbit primary antibody at 1:1000 (M20; Santa Cruz Biotechnology, Santa Cruz, CA) and an Alexa 568-conjugated goat anti-rabbit secondary antibody (Life Technologies, Grand Island, NY) at a 1:1000 dilution in 1% normal goat serum (Vector Laboratories, Burlingame, CA). Sections were washed in between incubations with 0.05 M Tris pH 7.5, 0.05 M NaCl, 0.3% Triton-X-100 (Fisher Scientific, Pittsburgh, PA). Sections were mounted on SuperFrost Plus slides (Fisher Scientific) and coverslipped with 50 mg/ml N-propyl gallate and 1 μ g/ml DAPI (4',6-Diamidino-2-phenylindole) in 0.2 M Tris, 90% glycerol, pH 7.5 (Sigma Aldrich, St. Louis, MO).

2.5. Data Analysis

GR deletion, defined as the absence of GR immunoreactivity, was mapped in the prefrontal cortex from 2.80 to 0.86 mm anterior to bregma [24]. Images were captured on an Olympus BX50 fluorescence microscope (Center Valley, PA, USA) using the same exposure settings for all mice. Open field, forced swim, and plasma corticosterone data were analyzed by 2-way ANOVA for the main effects of PFC GR deletion and antidepressant treatment. Post-hoc comparisons were performed where main effects were significant by t-test with Bonferroni correction for multiple comparisons. Data are expressed as mean $\pm$ SEM; significance was defined as $P < 0.05$.

3. Results

Virally-transduced GR deletion was observed in some mice as far anterior as the prelimbic cortex at bregma +2.80 mm or as far posterior as the cingulate cortex at bregma +0.86 mm. Figure 2 diagrams the smallest and largest extent of GR deletion, with representative photos of virally-mediated GR deletion shown in Figure 3. GR loss did not occur outside of virally infected cortex (Figure 3, right). Deletion was most extensive in the prelimbic PFC but extended into a portion of the infralimbic PFC in some mice, particularly at more anterior levels. However, the region of GR deletion common to all mice injected with the AAV2/9-Cre vector was limited for the most part to the prelimbic cortex and anterior cingulate gyrus in the most medial aspect of the PFC. DAPI staining did not qualitatively indicate any detectable cell loss associated with GR deletion (not shown). Because of some anatomical

variability in the extent of GR loss throughout the extent of the PFC, GR deletion is referred to as GR knockdown (PFC-GRKD).

There were no significant effects of PFC GR knock-down or imipramine treatment on total activity in an open field (Table 1). The large error in open field activity for the PFC-GFP group treated with 30 mg/kg imipramine was associated with a single mouse that exhibited a high level of activity that was 1.7 standard deviations above the group mean.

Immobility during a forced swim exhibited a significant main effect of imipramine treatment ($F_{1,24} = 3.406$; $P = 0.049$) but not PFC GR knock-down. However, only PFC-GRKD mice exhibited significant decreases in immobility after imipramine treatment. In this group, treatment with 30 mg/kg imipramine significantly reduced immobility compared to the effects of saline treatment (Figure 4, top). The high level of open field activity of one mouse (see above) prompted us to evaluate if differences in forced swim activity might be related to overall activity. However, there was no correlation between activity in the forced swim test and activity in the open field test (not shown).

Because our prior experiments primarily showed effects of imipramine on stimulated HPA activity [25], we examined plasma corticosterone after the stress of the 5-min forced swim to evaluate the impact of PFC GR knock-down on HPA regulation after imipramine treatment. Plasma corticosterone responses to the stress of a forced swim exhibited a significant main effect of imipramine treatment ($F_{1,24} = 12.814$; $P = 0.0002$) but not GR knock-down. Only PFC-GFP mice showed significant effects of imipramine on plasma corticosterone at the post-hoc level, with levels in PFC-GFP mice treated with 30 mg/kg imipramine being significantly higher than those in PFC-GFP mice treated with either saline or 15 mg/kg imipramine (Figure 4, bottom). Although similar trends were evident in PFC-GRKD mice, differences were not significant after correction for multiple comparisons.

There was no evident relationship between the anatomical extent of PFC GR knock-down and immobility or corticosterone data. Mice with the most restricted GR loss had levels of forced swim immobility and post-swim plasma corticosterone that were similar to those in mice with greater lateral or anterior-posterior GR loss.

4. Discussion

We have shown that PFC GR knock-down increases behavioral sensitivity to antidepressants without affecting overall locomotor activity. PFC GR knock-down did not markedly affect immediate plasma corticosterone responses to swim stress, at least at the time point studied. Although we did not attempt to target regions of the prefrontal cortex separately, similar results occurred in mice with GR loss concentrated in the medial prefrontal and dorsomedial anterior cingulate cortex, suggesting that these regions may be sufficient for the effects we observed.

PFC GR knock-down did not alter baseline immobility or general activity levels, but did enhance the ability of chronic imipramine treatment to decrease immobility in the forced swim test. The dose and duration of imipramine treatment was comparable to that we have found to affect behavior and GR expression in previous studies [6, 25]. Our results suggest

that PFC GR in the areas covered by our injections can interfere with the effects of antidepressants such as imipramine. Consistent with our results, mice with deletion of forebrain GR, including PFC GR, are more likely than their floxed GR counterparts to respond to chronic imipramine treatment with significant decreases in immobility [26].

We did not observe any effect of PFC GR knock-down on corticosterone responses to the stress of forced swim. This finding is at odds with the results of medial PFC GR knockdown and glucocorticoid implant studies, which in combination indicate that medial PFC GR inhibit responses to acute stress [8, 11]. Our negative data in this regard could be due to number of mice or to the number or timing of blood samples, since we sampled at 5 min, whereas significant differences in prior studies were observed at least 20 min after the start of stress. We also found that higher doses of imipramine increased plasma corticosterone levels elicited by the stress of a forced swim, agreeing with our prior findings that imipramine can enhance stimulated HPA activity [25]. Although inhibitory effects of antidepressant treatment on HPA activity have been reported, the literature is not consistent in this regard [23, 25, 27].

Because our previous data indicated that antidepressant effects on PFC GR expression were distributed across different PFC subdivisions [6, 7], we did not initially target individual regions of the prefrontal cortex. Selective knockdown of infralimbic vs. prelimbic PFC GR was reported during the completion of the current experiments to have differential effects on emotional behavior and HPA activity, with only infralimbic GR loss increasing depression-like behavior and augmenting the impact of chronic stress on HPA responses to a novel stimulus [11]. However, data from mice in our study with more restricted GR loss are consistent with the lack of effect of prelimbic GR deletion on depression-like immobility [11], and suggest some localization of the effects we observed. Mice with GR deletion limited to the medial prelimbic and dorsal anterior cingulate cortex were present in all groups, and had similar immobility and corticosterone levels to mice with more extensive deletion in the same group. Thus, although we cannot make any distinctions between the roles of different PFC subdivisions, it seems likely that GR loss from the dorsomedial PFC is sufficient to increase sensitivity to the anti-immobility effects of antidepressants. This interpretation is consistent with the finding that deep brain stimulation of the prelimbic but not infralimbic cortex has antidepressant-like behavioral effects in rats [28], and with reports that activity in the rostral anterior cingulate, the human homologue of the prelimbic and anterior cingulate cortices [29], correlates with antidepressant response in depression [1].

The ability of PFC GR to interfere with antidepressant action might seem to conflict with two prior findings in the literature: (1) our previous report that imipramine increases PFC GR gene expression [6], an effect that might be predicted from the current study to counteract the behavioral effects of imipramine, and (2) the recent report that loss of infralimbic GR promotes rather than reduces depression-like behavior [11]. Nevertheless, it is plausible that each of these results represents effects on separate PFC populations. For example, imipramine might induce GR expression in a separate population of PFC cells from that in which GR loss augments the behavioral effects of imipramine. Along the same lines, GR-expressing cells influencing antidepressant-induced changes in behavior might differ from those regulating baseline immobility, with baseline immobility being sensitive to

infralimbic GR but antidepressant-induced changes in immobility being responsive to GR in the more dorsal aspects of the medial PFC. It is also possible that glial vs. neuronal deletion of GR influences the effects of PFC GR knock-down, since we observed virtual elimination of GR immunoreactivity, whereas relative sparing of glial GR expression occurred in the studies demonstrating antidepressant effects of infralimbic GR knockdown [11].

Despite disagreement as to the correspondence between anatomical divisions of the rodent and human prefrontal cortex, several characteristics of the dorsomedial PFC make it a logical target for antidepressants. Nerve terminals in the prelimbic and anterior cingulate cortex express norepinephrine and serotonin transporters [30–34], which would be sensitive to antidepressants such as imipramine that inhibit norepinephrine or serotonin reuptake [35]. Consistent with their probable sensitivity to antidepressants, the prelimbic and anterior cingulate cortex have been reported to exhibit less swim stress-induced activation after antidepressant treatment [36, 37]. The prelimbic and anterior cingulate cortices are connected with the basolateral amygdala, ventral tegmental area, and dorsal raphe nucleus [38], which have respective roles in the expression of fear, anhedonia, and helplessness behaviors relevant to clinical depression [39–41]. The prelimbic PFC has been proposed to exert facilitative effects on fear expression [42], while the anterior dorsal cingulate cortex has been shown to be important for acquisition and retention of aversive memories [39]. Thus, the prelimbic and dorsal anterior cingulate cortices are relevant to mood disorders by virtue of their connectivity, sensitivity to negative emotional stimuli, and antidepressant responsiveness.

In conclusion, we have found that PFC GR knock-down has a novel effect to enhance behavioral sensitivity to antidepressants such as imipramine. GR loss in the dorsomedial prefrontal cortex, including the prelimbic and dorsal anterior cingulate cortex, is likely to be sufficient for this effect. Although our group sizes were small, our results are consistent with other literature localizing antidepressant responses to dorsomedial aspects of the prefrontal cortex [1, 28, 36, 37]. Antidepressants are currently the most common therapy for depression and anxiety [4]. Since glucocorticoid secretion is also often abnormal in depression and anxiety disorders [4], identifying the neurons and circuits in which PFC GR loss augments antidepressant efficacy could help to improve treatment for these diseases.

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Highlights

Prefrontal cortex glucocorticoid receptor knockdown enhances imipramine sensitivity

Prefrontal cortex glucocorticoid receptor knockdown does not affect general activity

Prefrontal cortex glucocorticoid receptor knockdown does not alter early adrenocortical axis responses to stress

Figure 1.

Timeline of experimental manipulations described in Methods. Mice were tested at the end of 6 weeks of daily treatment with either saline or 15 or 30 mg/kg imipramine. The forced swim test consisted of a 15 min unscored swim followed 24 h later by a 5 min swim that was scored for immobility behavior. Blood was collected for plasma corticosterone (Cort) immediately after the 5 min swim. Mice underwent dexamethasone injection, lethal anesthesia, and transcardiac perfusion on day 42 after the 5 min swim, as described in Methods.

Figure 2.

Diagram of the extent of PFC GR deletion from +2.58 to +1.54 mm from bregma in floxed GR mice injected with AAV2/9-Cre (PFC-GRKD). Areas shaded in dark gray indicate the smallest extent of GR deletion, whereas light gray areas indicate the greatest extent of deletion. Diagrams are adapted from the Franklin and Paxinos mouse brain atlas [24], with the anterior commissure and corpus callosum indicated in black. Dotted lines show the midline and the boundaries of the cingulate gyrus, prelimbic cortex, and infralimbic cortex, respectively, from top to bottom in each diagram (note that infralimbic cortex starts at +2.10 from bregma in this atlas).

Figure 3.

Representative examples of GR gene deletion captured at 400X magnification in mice injected with AAV2/9-GFP (PFC-GFP) and AAV2/9-Cre vectors (PFC-GRKD). GR immunofluorescence is shown in the prefrontal cortex of a PFC-GFP mouse (left), the prefrontal cortex of a PFC-GRKD mouse (middle) and in the anterior motor cortex of a PFC-GRKD mouse outside of the infected area delineated by GFP expression (right; PFC-GRKD, uninfected cortex). All mice were treated with 10 mg/kg dexamethasone 1 h before death to enhance GR nuclear localization. Images were captured in sections at approximately +1.94 from bregma, based on the mouse brain atlas of Franklin and Paxinos [24]. Scale bar in middle panel is 50 μ m.

Figure 4.

Immobility during (top) and plasma corticosterone immediately after (bottom) a 5 min forced swim in PFC-GFP and PFC-GRKD mice treated daily for six weeks with either saline (white bars) or 15 (gray bars) or 30 (black bars) mg/kg imipramine. Group *N*s are shown within the data bars of the lower graph and are the same for immobility and corticosterone. *, $P < 0.05$ vs. saline in the same virus group; #, $P < 0.05$ vs. 15 mg/kg imipramine in the same virus group.

Table 1

Total distance (cm) traveled in an open field by PFC-GFP and PFC-GRKO mice after 6 weeks treatment with saline or 15 or 30 mg/kg/d imipramine. Group sizes are indicated in parentheses.

	<u>PFC-GFP</u>	<u>PFC-GRKO</u>
Saline	544 ± 82 (5)	669 ± 108 (6)
15 mg/kg imipramine	468 ± 95 (5)	460 ± 58 (6)
30 mg/kg imipramine	722 ± 355 (4)	600 ± 144 (5)