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Short communication

Differential effects of glucocorticoid and mineralocorticoid antagonism on anxiety behavior in mild traumatic brain injury

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HIGHLIGHTS

- A novel rat model of social stress paired with mild brain injury was utilized.
- Glucocorticoid receptor antagonism prevented anxiety induced by mild TBI.
- Mineralocorticoid receptor blockade less effective at preventing mild TBI anxiety.
- Results suggest targeting glucocorticoid receptors to treat symptoms of mild TBI.

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ABSTRACT

Mild traumatic brain injuries (TBIs) comprise three-quarters of all TBIs occurring in the United States annually, and psychological symptoms arising from them can last years after injury. One commonly observed symptom following mild TBI is generalized anxiety. Most mild TBIs happen in stressful situations (sports, war, domestic violence, etc.) when glucocorticoids are elevated in the brain at the time of impact, and glucocorticoids have negative effects on neuronal health following TBI. Therefore, blocking glucocorticoid receptors might prevent emergence of anxiety symptoms post-injury. Adult male rats received mifepristone (20 mg/kg) or spironolactone (50 mg/kg) to block glucocorticoid and mineralocorticoid receptors, respectively, 40 min prior to being exposed to acute social defeat stress followed immediately by mild TBI. In defeated rats with concomitant mild TBI, mifepristone restored time spent in the open arms of an elevated plus maze to control levels, demonstrating for the first time that glucocorticoid receptors play a critical role in the development of anxiety after mild TBI. Future treatments could target these receptors, alleviating anxiety as a major side effect in victims of mild TBI sustained in stressful situations.

Mild traumatic brain injuries (TBIs) are common occurrences in sports, warfare and everyday life, and comprise 75–80% of total TBIs sustained each year [1]. Mild TBIs are non-penetrating impacts to the head that result in either a brief (<30 min) loss of consciousness or an otherwise altered mental status [1,2]. While many who sustain a mild TBI fully recover within a few months, up to 60% continue to experience post-concussive symptoms for months or years after impact [1]. Symptoms often include generalized anxiety and post-traumatic stress disorder (PTSD)-like behaviors [1,3,4], which are mediated by brain regions such as the amygdala and the hippocampus [5]. These parts of the limbic system are particularly vulnerable to mechanical forces involved in mild TBI [1], and to cellular damage [5].

Many mild TBIs occur in high-energy, stressful situations, such as combat, domestic abuse, traumatic accidents or sporting environments [1,2,6]. In such a state of arousal, glucocorticoids such as cortisol (corticosterone in rats) are elevated [7]. These steroids can bind to both glucocorticoid (GR) and mineralocorticoid (MR) receptors, found widely throughout the limbic system [8]. MRs have a higher affinity for cortisol/corticosterone than do glucocorticoid receptors (GRs) [8], thus MRs become saturated as glucocorticoid levels rise initially, with GRs becoming bound at higher concentrations. Activation of both receptors elicits changes in ion regulation and in neurotransmitter release and function [8].

There is some indirect evidence to suggest that glucocorticoids play a role in psychological symptoms induced by mild TBI. For

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example, GR antagonism has been shown to prevent neuron loss in the hippocampus after TBI in rats [9], a region known to be damaged by mild TBI and associated with resulting psychological symptoms [5]. Psychological stress at the time of injury may be partly indicative of the extent of post-mild TBI impairment [6]. The GR antagonist mifepristone was also shown to be significantly better than placebo at treating symptoms of PTSD in military veterans [10]. Antagonism of MRs prior to injury has been shown in rats to reduce apoptotic damage in the hippocampus 24 h after impact [9]. Furthermore, antagonism of either GRs or MRs has been shown to increase the amount of time spent in the open arms of an elevated plus maze in pre-stressed rats [8]. Notably, anxiety-like behavior in rats following a mild TBI concurrent with stress is more pronounced than after injury alone [7]. The ability of GR or MR antagonism to protect against neuronal injury caused by TBI, combined with the anxiolytic effects of GR and MR blockade following stress, suggests that early targeting of one or both types of receptors may potentially abate anxiety resulting from stress-associated mild TBI.

Therefore, this experiment aimed to determine whether MRs or GRs play a differential role in post-mild TBI anxiety behaviors, via blockade of each receptor type. This was tested in a relevant rodent model of psychosocial stress with mild TBI, which reliably increases plasma corticosterone at the time of injury and elicits heightened anxiety-like behavior to a greater extent than either stress or mild TBI alone [7]. We show for the first time that GR antagonism is effective in reducing generalized anxiety caused by mild TBI incurred during stressful events.

These experiments were approved by the Institutional Animal Care and Use Committee of the University of South Dakota and all efforts were made to minimize the number of animals used and potential suffering. Forty-nine adult (8–10 weeks old) male Sprague-Dawley rats were housed on a reverse light cycle (12L:12D, lights off 10 AM) with food/water available *ad libitum*. All behavioral testing was performed under red lighting and commenced one hour after the dark cycle began.

Rats were acclimated to a solitary cage and the testing room (in preparation for social defeat) for 40 min/day for three days prior to the treatment day [7]. On the treatment day, rats received a single subcutaneous (sc.) injection of either vehicle (propylene glycol, 1 mL/kg), the MR antagonist spironolactone (50 mg/kg [11]), or the GR antagonist mifepristone (20 mg/kg [11]), all purchased from Sigma-Aldrich (St. Louis, MO, United States), 40 min before exposure to either control (no stress/brain injury) or stress + mild TBI treatment (n = 7-10 per group). In a follow up study to determine whether any of the positive effects of mifepristone treatment in the stress/injury group were due to GR antagonism of the stress experience regardless of head injury, rats were treated with either mifepristone (20 mg/kg) or vehicle 30 min prior to social defeat and sham surgery (n = 6-7 per group). Drug concentrations were chosen based on efficacy in reducing anxiety-like behaviors in pre-stressed rats [11].

Forty minutes following vehicle or GR or MR antagonism, stress/injury or stress/sham rats underwent a single episode of social defeat by a larger, aggressive male rat (> 100 g larger than subject), which reliably elevates plasma corticosterone [7] to create the desired conditions for mild TBI incurred under a stressed state. Control rats were placed in an empty cage similar to acclimation days (see Davies et al., 2016, [7] for protocol details).

Details of the surgical procedure to induce mTBI are given in Meyer et al. (2012) [5]. Details regarding the social defeat model coupled with the weight drop mild TBI are outlined in Davies et al. (2015) [7]. Briefly, at the conclusion of the defeat trial, rats were anesthetized with isoflurane (3–4% in 3.0 L/min O_2). The skin on the top of the head was shaved, disinfected, and a 1-inch long incision was made to expose the skull. A calibrated weight-drop device then delivered a 5477 N/m^2 impact to the exposed skull of the rat immediately behind bregma using a 175 g weight dropped from 42 cm, with the force distributed across a 10 mm diameter area via a vertical transducer rod positioned just posterior to bregma and centered over the intraparietal suture. Induction of a mild traumatic brain injury was confirmed via delayed righting-reflex time post-surgery and by the absence of more severe damage (skull fracture, gross brain or vascular damage) at the conclusion of behavioral testing [5]. Control (sham) animals underwent surgery without receiving an impact.

Eight to nine days post-surgery, rats were evaluated for generalized anxiety using an elevated-plus maze (EPM) as described previously [5]. Rats were placed in the center of the maze facing the closed arms and allowed to explore freely for 5 min. Time spent in the open arms and total distance moved in the maze were measured using Ethovision XT V5.1 (Noldus Information Technology, Leesburg, VA USA).

A *t*-test compared righting reflex times and duration of time spent under anesthesia between control and defeat/mild TBI animals that had received vehicle. Two-way ANOVA (treatment x drug) were used to analyze time spent in the open arms of the EPM, as well as distance traveled in the maze, with *a priori* comparisons made by one-way ANOVA or Student–Newman–Keuls (SNK) tests for multiple pairwise comparisons. One way ANOVA with SNK tests were used to compare the intensity of the social defeat event across the three drug treatment groups. For animals that experienced defeat without concussive injury, a *t*-test compared those treated with vehicle versus those that received mifepristone. The significance level was set at $P < 0.05$ throughout, and all analyses were performed using SigmaStat v11.0.

Vehicle-treated rats with mild TBI had a delayed righting reflex compared to vehicle-treated rats that underwent sham surgery ($t_{16} = 2.396$, $P = 0.029$; Fig. 1A), demonstrating presence of mild TBI [5]. With regard to EPM testing, there was a significant negative effect of treatment on time spent in open arms ($F_{2,41} = 4.930$; $P = 0.032$). Furthermore, *a priori* pair-wise comparisons revealed that vehicle-treated rats subjected to defeat/mild TBI spent less time in open arms compared to vehicle-treated controls (SNK $P = 0.028$), an effect that was not apparent in the mifepristone (SNK $P = 0.855$) or spironolactone (SNK $P = 0.144$) groups (Fig. 1B). Subsequent one-way ANOVA to examine drug effects within injury group revealed no effect of drug in the control group ($F_{2,20} = 0.004$; $P = 0.996$), but a drug effect in the defeat/mild TBI group ($F_{2,22} = 3.772$; $P = 0.039$) (Fig. 1B). Rats treated with mifepristone prior to defeat/mild TBI showed greater time in the open arms as compared to vehicle-treated rats (SNK $P = 0.031$; Fig. 1B). However, time spent in open arms did not differ between mifepristone and spironolactone injury groups nor between vehicle and spironolactone injury groups (SNK $P > 0.05$; Fig. 1B). These effects could not be accounted for by differences in time spent under isoflurane (control, mean+/− SEM = 18.3 min +/− 0.59; defeat/mild TBI, mean+/− SEM = 19.2 min +/− 0.44, $t_{47} = 1.170$, $P = 0.248$). Similarly, intensity of the social defeat episode did not differ among rats that received either vehicle, mifepristone, or spironolactone ($n = 26$, $F_{1,20} = 0.256$, $P = 0.777$, data not shown), nor was there any difference in distance moved across all groups (drug, $F_{2,43} = 1.034$; $P = 0.364$, injury $F_{1,43} = 2.336$; $P = 0.134$, drug x treatment $F_{2,43} = 0.360$; $P = 0.700$; Fig. 1C). In a follow-up study to address the possibility that it is not the effects of social defeat experience alone that are affected by the GR antagonist, animals that received social defeat, but not mild TBI exhibited similar times in open arms ($t = 0.761$, $P > 0.05$) when treated with vehicle ($n = 7$, mean = 16.6 s ± SEM 10.1) and mifepristone ($n = 6$, mean = 7.6 s ± SEM 4.8).

Our results indicate that the GR antagonist mifepristone was able to restore anxiety-like behavior to control levels in animals that had experienced social defeat concurrent with mild TBI. While MR antagonism prior to injury partially appeared to reverse