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Role of 5-HT_{2A} receptors in the stress-induced down-regulation of brain-derived neurotrophic factor expression in rat hippocampus

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Abstract

Immobilization stress decreases the expression of BDNF mRNA in the rat hippocampus, and this effect could contribute to the atrophy of hippocampal neurons. This study examines the influence of selective 5-HT, as well as norepinephrine, receptor antagonists on the stress-induced down-regulation of BDNF mRNA. Pretreatment with a selective 5-HT_{2A} receptor antagonist, MDL100,907, significantly blocked the influence of stress on expression of BDNF mRNA. In contrast, pretreatment with either a selective 5-HT_{2C} or 5-HT_{1A} receptor antagonist did not influence the stress-induced decrease in levels of BDNF mRNA. The stress-induced decrease was also not influenced by pretreatment with antagonists of $\beta_{1/2}$- or α_1-adrenergic, or CRF-R1 receptors. The results demonstrate that 5-HT_{2A} receptors mediate, at least in part, the stress-induced down-regulation of BDNF expression in the rat hippocampus.

Keywords: Brain derived neurotrophic factor; Stress; Serotonin; 5-HT_{2A}; 5-HT_{2C}; Hippocampus

Prolonged, severe stress is thought to play an important role in precipitating and exacerbating several psychiatric illnesses, including depression and posttraumatic stress disorder (PTSD). Exposure to a stressful stimulus leads to the activation of several neurotransmitter and neuroendocrine systems [2,3,8] of which the hypothalamo-pituitary-adrenocortical (HPA) axis is the key hormonal component. Activation of the HPA axis leads to the secretion of glucocorticoids which play an important role in preparing the animal to adapt to stress and also provide a negative feedback regulation of the axis [4,6]. The hippocampus is one of the major suprahypothalamic sites which mediates negative feedback regulation of the HPA axis [6]. The hippocampus is also susceptible to stress-induced damage. Animals exposed to chronic stress show hippocampal neuronal atrophy and death, and recent reports suggest that similar effects are observed in patients suffering from recurrent major depression and PTSD [1,2,12,13].

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The recent finding that stress decreases the expression of BDNF within the hippocampus suggests that regulation of neurotrophins could also contribute to the effects of stress on neuronal survival and function [14]. In adrenalectomized rats the influence of stress on the down-regulation of BDNF was not significantly altered suggesting that glucocorticoids alone could not fully explain the regulation of BDNF by stress [14]. There are several neurotransmitter systems, including monoamine systems, that are influenced by stress and that could regulate the expression of BDNF [2,8]. One of these is the serotonergic system, which is profoundly influenced by stress [2,7,11,16]. Moreover, recent work has shown that activation of 5-HT_{2A} receptors decreases levels of BDNF mRNA in the hippocampus in a manner similar to that observed after stress. This finding raises the possibility that the stress-induced down-regulation of BDNF may be mediated by release of 5-HT and activation of 5-HT_{2A} receptors. In a preliminary study, we found that pretreatment with a non-selective 5-$HT_{2A/2C}$ receptor antagonist, ketanserin, partially blocked the effects of stress on BDNF expression [17].

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The present study further characterizes the role of the 5-HT_{2A} versus the 5-HT_{2C} receptor subtype in the regulation of BDNF mRNA by stress. In addition, the role of other monoaminergic neurotransmitter as well as CRF receptors in mediating the stress-induced down-regulation of BDNF mRNA is examined.

Male Sprague–Dawley rats (170–210 g) (CAMM, Wayne, NJ) were group housed and maintained on a 12:12 h light-dark cycle with access to food and water ad libitum. Stress is administered by placing rats in plastic immobilization bags (Harvard Instruments) for 2 h. Treatment groups were as follows; Experiment 1: vehicle/sham, vehicle/stress, 1 mg/kg MDL 100,907/stress; Experiment 2: vehicle/sham, vehicle/stress, 15 mg/kg SB 206553/stress, 1 mg/kg prazosin/stress; Experiment 3: vehicle/sham, vehicle/stress, 0.5 mg/kg WAY 100635/stress, 10 mg/kg CP 154,526/stress; Experiment 4: vehicle/sham, vehicle/stress, 10 mg/kg propanolol/stress. Sprague–Dawley rats (250–280 g) were administered vehicle (0.9% saline) or receptor antagonist 30 min prior to stress, rats were then subjected to immobilization stress for 2 h. To study the effects of the drugs on basal expression of BDNF mRNA, animals were administered vehicle or 1 mg/kg MDL 100,907, 15 mg/kg SB 206553, 0.5 mg/kg WAY 100635, 10 mg/kg propanolol, 1 mg/kg prazosin, and 10 mg/kg CP 154,526. The number of animals for all experiments is 3–4 per group.

Animals were decapitated immediately following stress or drug treatments. All animal use procedures were in strict accordance with the guidelines of the National Institutes for the Care and Use of Laboratory Animals and were approved by the Yale Animal Care and Use Committee. In situ hybridization and quantitation of BDNF mRNA was carried out as previously described [17]. Results were subjected to analysis of variance (ANOVA) followed by the Newmann-Keuls post-hoc test with a significance level of $P < 0.05$.

In a preliminary study we found that pretreatment with ketanserin, a non-selective 5-$HT_{2A/2C}$ receptor antagonist, led to a partial, but highly significant blockade of the stress-induced down-regulation of BDNF mRNA in the dentate gyrus granule cell layer [17]. To further characterize the 5-HT_2 receptor subtype involved in mediating the effects of stress on BDNF expression more selective antagonists were utilized. Pretreatment with MDL 100,907, a 5-HT_{2A} selective antagonist, lead to a partial but highly significant blockade of the stress-induced down-regulation of BDNF mRNA in dentate gyrus (Fig. 1). In this experiment stress also significantly decreased BDNF mRNA in the CA3 pyramidal cell layer and this effect was blocked by pretreatment with MDL 100,907. In subsequent studies, levels of BDNF mRNA in CA3 tended to be reduced by stress, although the decrease did not reach statistical significance. This less robust effect of stress in the CA3 pyramidal cell layer has been observed in a previous report [17]. SB 206553, a 5-HT_{2C} receptor selective antagonist, failed to block the stress-induced regulation of BDNF expression (Fig. 2), indicating that it is the 5-HT_{2A} subtype that mediates the ability of ketanserin to block the effects of stress on BDNF mRNA levels. Administration of either MDL 100,907 or SB 206553 alone did not influence the expression of BDNF mRNA in either the dentate gyrus granule or CA3 pyramidal cell layers when compared to vehicle treated controls (MDL 100,907: dentate gyrus, 102 ± 2; CA3, 105 ± 7; SB 206553: dentate gyrus, 109 ± 4; CA3, 106 ± 8; values are expressed as % of vehicle and are the mean ± SEM, $n = 4$).

The role of the 5-HT_{1A}, α_1-adrenergic, $\beta_{1/2}$-adrenergic, and the CRF-R1 receptors in the stress regulation of BDNF expression was also examined. WAY 100635, a 5-HT_{1A} antagonist, did not block the decrease in BDNF mRNA in the dentate gyrus following stress (Table 1), suggesting that the influence of 5-HT released during stress on hippocampal BDNF expression may be predominantly mediated by the 5-HT_{2A} receptor. Considerable evidence has demonstrated that the noradrenergic system is an important player in stress responses [8,16]. However antagonists of α_1- or $\beta_{1/2}$-adrenergic receptors did not influence the effects of stress on BDNF mRNA (Table 1). A non-peptide corticotrophin releasing factor (CRF) receptor antagonist, CP 154,526, with greater affinity for the CRF-R1 receptor over CRF-R2, did not significantly influence the stress-

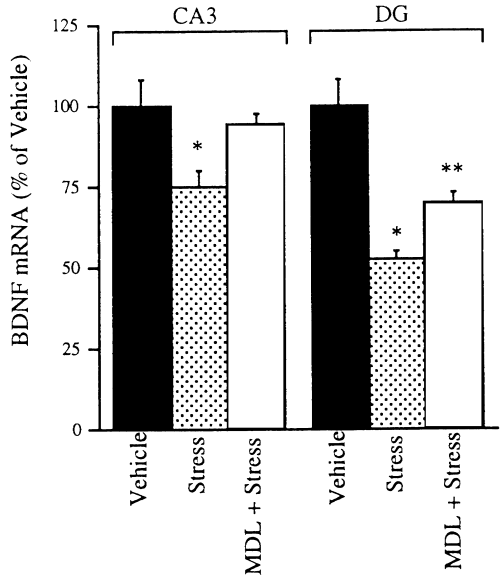

Fig. 1. Pretreatment with a selective 5-HT_{2A} receptor antagonist blocks the stress-induced down-regulation of BDNF. Rats were pretreated with vehicle or MDL100,907 (MDL) (1 mg/kg) 30 min prior to being subjected to immobilization stress (2 h). Results are expressed as percent of vehicle and are the mean ± SEM ($n = 4$). Analysis of variance comparing BDNF levels in the dentate gyrus of the control, stress, and MDL + stress groups revealed a statistically significant difference ($F(2,9) = 20.58$, $P < 0.0005$). Newman-Keuls post-hoc comparison revealed a significant difference between stress and control ($^*P < 0.001$), stress and MDL + stress ($^{**}P < 0.05$), and control and MDL + stress ($^{**}P < 0.01$). Analysis of variance comparing BDNF levels in the CA3 pyramidal cell layer of the control, stress, and MDL + stress groups revealed a statistically significant difference ($F(2,9) = 5.04$, $P < 0.05$). Newman-Keuls post-hoc comparison revealed a significant difference between stress and control ($^*P < 0.05$).

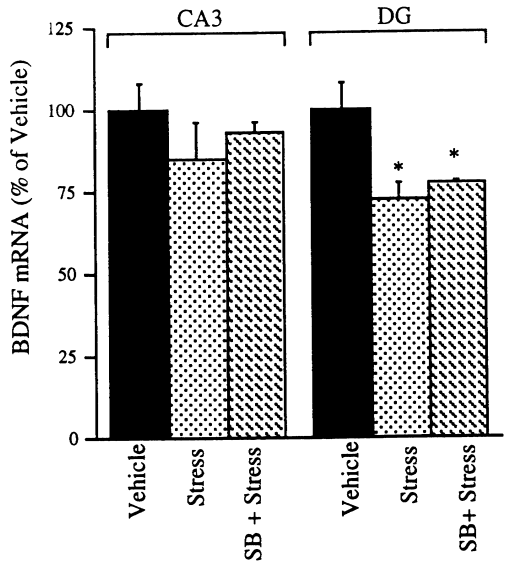

Fig. 2. Pretreatment with a selective 5-HT_{2C} receptor antagonist does not block stress-induced down-regulation of BDNF. Rats were pretreated with vehicle or SB 206553 (SB) (15 mg/kg) 30 min prior to being subjected to immobilization stress (2 h). Results are expressed as percent of vehicle and are the mean ± SEM ($n = 4$). Analysis of variance comparing BDNF levels in the dentate gyrus of the control, stress, and SB + stress groups revealed a statistically significant difference ($F(2,9) = 4.85$, $P < 0.05$). Newman-Keuls post-hoc comparison revealed a significant difference between stress and control (*$P < 0.05$) and control and SB + stress (*$P < 0.05$).

induced decrease in BDNF mRNA. Administration of the antagonists alone did not significantly influence the basal expression of BDNF mRNA in the hippocampus (data not shown).

Recent studies have demonstrated that stress decreases the expression of BDNF in hippocampus, particularly in the dentate gyrus granule cell layer [14]. The down-regulation of BDNF by stress is not blocked by adrenalectomy, indicating that factors other than adrenal-glucocorticoids mediate this effect [14]. Levels of 5-HT in the hippocampus are increased in response to several different types of stress, including immobilization stress [2,7,11,16]. Based on this information and the recent finding that activation of 5-HT_{2A} receptors also down-regulates BDNF mRNA in hippocampus, we hypothesized that this 5-HT receptor subtype may contribute to the influence of stress on BDNF expression. The results of this study demonstrates that pretreatment with the selective 5-HT_{2A} receptor antagonist, MDL 100,907, leads to a partial, but significant blockade of the stress-induced decrease in BDNF expression. Pretreatment with SB 206553, a selective 5-HT_{2C} receptor antagonist, or WAY 100635, a selective 5-HT_{1A} receptor antagonist, did not influence the stress-induced decrease in BDNF mRNA expression. It appears that serotonin release during stress and activation of the 5-HT_{2A} receptor may be one of the mechanisms via which stress influences BDNF expression.

Partial blockade of the stress response by the 5-HT_{2A} receptor implies that other neurotransmitter systems are involved. The noradrenergic neuronal pathways are powerfully influenced by stress and norepinephrine is reported to regulate the expression of BDNF [8,15,16]. However pretreatment with prazosin, an α_1-adrenergic receptor antagonist, or propanolol, a $\beta_{1/2}$-adrenergic receptor antagonist, did not influence the stress regulation of BDNF expression. The CRF neuropeptide system and the CRF-R1 receptor subtype are known to mediate many central effects of stress [6]. In the present study, pretreatment with the CRF-R1 receptor antagonist, CP 154,526, did not block the stress-induced decrease in BDNF mRNA, indicating that this receptor subtype is not involved in the stress effect. It is possible that the CRF-R2 receptor subtype could play a role in the regulation of BDNF by stress, although there are currently no selective antagonists for this receptor.

The mechanism responsible for the down-regulation of BDNF mRNA in the hippocampus by stress may be explained by the results of this and previous studies [2,7,11,16,17]. Serotonergic inputs from the dorsal raphe are thought to exert a global control over the hippocampus via modulation of local inhibitory interneurons [5]. 5-HT released into the hippocampus during immobilization stress could activate 5-HT_{2A} receptors expressed on GABAergic interneurons and increase GABA release (Fig. 3). Activation of 5-HT_{2A} receptors is reported to increase the firing of GABAergic neurons and to thereby induce IPSPs in granule cells [10]. Such an effect on the firing rate of granule cells could explain the finding that GABA decreases BDNF expression in the hippocampus [18]. Because the 5-HT_{2A} receptor antagonist only partially blocks the effect of stress, it is likely that some other neurotransmitter, neuroendocrine, or cytokine-immune factors are also involved in mediating the actions of stress on the expression of BDNF.

A role for the 5-HT_{2A} receptor in stress responses is also supported by a study demonstrating that ketanserin blocks

Table 1

Influence of receptor antagonists on stress regulation of BDNF mRNA. Rats were administered vehicle or antagonists and 30 min later were exposed to immobilization stress (2 h). BDNF mRNA was determined by in situ hybridization and quantified by densitometry. The results are expressed as % of vehicle and are the mean ± SEM ($n = 3$–4). *$P < 0.05$ compared to vehicle (ANOVA and Newman-Keuls post-hoc test).

	BDNF mRNA (% of vehicle)	
Treatment group	CA3	DG
Vehicle	100 ± 8	100 ± 7
Stress	90 ± 7	62 ± 4*
WAY100635 ± stress	95 ± 6	70 ± 1*
CP 154,526 ± stress	85 ± 3	65 ± 1*
Vehicle	100 ± 4	100 ± 3
Stress	92 ± 4	67 ± 4*
Propranolol ± stress	87 ± 3	68 ± 2*
Vehicle	100 ± 8	100 ± 8
Stress	85 ± 11	72 ± 5*
Prazosin ± stress	81 ± 14	72 ± 8*

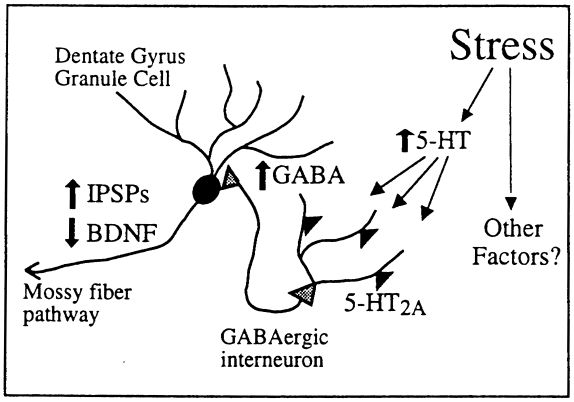

Fig. 3. Cellular model for stress-induced down-regulation of BDNF expression in hippocampus. This stress effect could occur via 5-HT_{2A} receptors located on GABAergic interneurons, which upon activation are known to increase IPSPs in the granule cells. The increased inhibitory control of dentate gyrus granule cells could lead to down-regulation of BDNF mRNA. Because the 5-HT_{2A} receptor antagonist does not completely block the stress-induced down-regulation of BDNF, it is likely that there are other factors regulated by stress that also contribute to this effect.

stress-induced changes in the expression of the gene transcription factors, NGF1-A and mineralocorticoid receptor [9]. This suggests that stress-induced changes in 5-HT lead to downstream regulation of at least three, if not more, stress responsive genes. Decreased expression of BDNF, as well as altered expression of NGF1-A and mineralocorticoid receptor, could contribute to the stress-induced atrophy and death of vulnerable hippocampal neurons. Based on the findings of this study it is reasonable to speculate that blockade of 5-HT_{2A} receptors could protect hippocampal neurons from damage. It fact, many types of antidepressants are known to antagonize or down-regulate 5-HT_{2A} receptors, and blockade of the stress-induced down-regulation of BDNF via this mechanism could contribute to the antidepressant effects of these agents.

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