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Involvement of the Endocannabinoid System in the Ability of Long-Term Tricyclic Antidepressant Treatment to Suppress Stress-Induced Activation of the Hypothalamic–Pituitary–Adrenal Axis

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The efficacy of antidepressants has been linked in part to their ability to reduce activity of the hypothalamic–pituitary–adrenal (HPA) axis; however, the mechanism by which antidepressants regulate the HPA axis is largely unknown. Given that recent research has demonstrated that endocannabinoids can regulate the HPA axis and exhibit antidepressant potential, we examined the hypothesis that the endocannabinoid system is regulated by long-term antidepressant treatment. Three-week administration of the tricyclic antidepressant desipramine (10 mg/kg/day) resulted in a significant increase in the density of the cannabinoid CB_1 receptor in the hippocampus and hypothalamus, without significantly altering endocannabinoid content in any brain structure examined. Furthermore, chronic desipramine treatment resulted in a reduction in both secretion of corticosterone and the induction of the immediate early gene c-fos in the medial dorsal parvocellular region of the paraventricular nucleus of the hypothalamus (PVN) following a 5 min exposure to swim stress. Acute treatment with the CB_1 receptor antagonist, AM251 (1 mg/kg), before exposure to swim stress, completely occluded the ability of desipramine to reduce both corticosterone secretion and induction of c-fos expression in the PVN. Collectively, these data demonstrate that CB_1 receptor density in the hippocampus and hypothalamus is increased by chronic tricyclic antidepressant treatment, and suggest that this upregulation could contribute to the ability of tricyclic antidepressants to suppress stress-induced activation of the HPA axis.

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INTRODUCTION

Major depression is a psychiatric disease that results in dramatic alterations in emotional, neurovegetative, and cognitive processes. The neurobiology of depression is not well understood; however, a large body of evidence convincingly demonstrates a critical role of the hypothalamic–pituitary–adrenal (HPA) axis (Holsboer, 2000). Specifically, both corticotrophin releasing hormone (CRH) and cortisol are reported to be increased in the cerebrospinal fluid and plasma of depressed patients (Arborelius *et al*, 1999; Parker *et al*, 2003; Holsboer, 2000). Furthermore, the ability of glucocorticoid hormones to exert negative feedback on HPA axis activity appears to be deficient in depression, resulting in a feed-forward hyperactivation of this system (Parker *et al*, 2003; Holsboer, 2000). This enhanced output of the HPA axis appears functionally relevant to depression, as long-term antidepressant treatment attenuates this phenomenon in humans (De Bellis *et al*, 1993; Pariante *et al*, 2004; Greden *et al*, 1983; Michelson *et al*, 1997), and suppresses stress-induced activation of the HPA axis in other species (Reul *et al*, 1993; Connor *et al*, 2000; Holsboer and Barden, 1996; de Medeiros *et al*, 2005; Butterweck *et al*, 2001; Stout *et al*, 2002). The ability of antidepressants to suppress HPA axis hyperactivity has been shown to be coupled to their clinical efficacy (Appelhof *et al*, 2006; Young *et al*, 2004). Specifically, normalization of glucocorticoid feedback and hypersecretion is associated with clinical remission, and patients who do not exhibit normalization of this system exhibit a significantly higher tendency to experience

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depressive relapse and have a poorer long-term prognosis (Ribeiro *et al*, 1993; Greden *et al*, 1983; Zobel *et al*, 2001). These data demonstrate that the ability of antidepressants to regulate the HPA axis could be integral to the remission of depressive symptoms; however, the mechanism by which antidepressants exert this effect is currently not well understood.

Given the role of the HPA axis in depression, it is interesting to note that recent work has suggested a critical role for the endocannabinoid system in regulating HPA axis activation. Specifically, electrophysiological studies have demonstrated that CB_1 cannabinoid receptors in the paraventricular nucleus of the hypothalamus (PVN) are located on glutamatergic terminals and gate excitatory activation of the CRH neurosecretory cells (Di *et al*, 2003). These data predict that activation of CB_1 receptors in the PVN would result in a suppression of HPA axis activity, whereas a disruption in endocannabinoid signaling would result in hyperactivity of the HPA axis. This hypothesis has received substantial support *in vivo*, as genetic or pharmacological disruption of endocannabinoid signaling results in exaggerated endocrine responses to stress, and conversely, inhibition of endocannabinoid uptake or metabolism attenuates stress-induced activation of the HPA axis (Patel *et al*, 2004; Barna *et al*, 2004).

As the endocannabinoid system can regulate HPA axis activity, and could play a role in both the pathophysiology and treatment of depression (Hill and Gorzalka, 2005a, b; Gobbi *et al*, 2005; Witkin *et al*, 2005), the present study was designed to examine whether chronic treatment with the tricyclic antidepressant desipramine regulates endocannabinoids and/or the CB_1 receptor.

METHODS

Subjects

Seventy-day-old male Sprague–Dawley rats (approx. 285 g at the onset of the study) housed in groups of three in triple wire mesh caging were used in this study. Colony rooms were maintained at 21°C, and on a 12 h light/dark cycle, with lights on at 0700. All rats were given *ad libitum* access to Purina Rat Chow and tap water. All treatments performed in this study were approved by the Animal Ethics Committee of the University of British Columbia and were consistent with the standards of the Canadian Council on Animal Care.

Treatment Procedure

For the biochemical studies, animals were divided into two treatment groups: one received 10 mg/kg desipramine (Sigma, Canada) in saline and the other an equivalent amount of saline alone. All subjects received daily intraperitoneal injections for 21 days; 18 h following the last injection, all subjects were rapidly decapitated. Prefrontal cortex (a tissue block composed of medial prefrontal cortex and anterior cingulate, which was anatomically defined as the area dorsal to the anterior olfactory nucleus and medial to the corpus callosum and claustrum formation), amygdala (composed of central, basolateral, and medial nuclei), hippocampus, and hypothalamus (a tissue block that was anatomically defined by a dorsal barrier of the top of the third ventricle and laterally by the striatum and fornix) were dissected out on ice, immediately frozen in liquid nitrogen, and stored at −80°C until analysis.

For neuroendocrine studies, animals were divided into four treatment conditions: (1) saline–vehicle (1 : 1 : 8 Tween 80 : dimethyl sulfoxide : 0.9% saline); (2) 10 mg/kg desipramine–vehicle; (3) saline–1 mg/kg AM251 (Tocris-Cookson, USA); (4) 10 mg/kg desipramine–1 mg/kg AM251. AM251 was administered in the Tween vehicle and desipramine was administered in saline. All injections were given intraperitoneally in a volume of 1 ml/kg using 26 1/2″ gauge needles. Rats were administered vehicle or 10 mg/kg desipramine injections for 21 days; on the 22nd day, the final injection of vehicle or desipramine was immediately preceded by an injection of 1 mg/kg AM251 or vehicle. Two cohorts of animals were prepared in these treatment conditions. One cohort was exposed to swim stress; the second cohort of animals was not exposed to swim stress to permit examination of the effects of these treatment conditions on basal activity of the HPA axis. At 1 h after the final injections, subjects were exposed to a 5 min swim stress session, which was performed in a cylindrical Plexiglas container, filled to a height of 30 cm with water at 21°C. At 45 min following stressing, subjects were subjected to a brief tail bleed to obtain blood for analysis of plasma corticosterone. At 1 h following the tail bleed, all subjects were overdosed with sodium pentobarbital (120 mg/kg) and trans-cardially perfused with 4% paraformaldehyde. The brains were then fixed in paraformaldehyde overnight and stored in phosphate-buffered saline until sectioned for immunohistochemical analysis. These time points were based on previous studies in which the peak corticosterone secretion and expression of c-fos following exposure to the swim stress were determined (Connor *et al*, 2000; Duncan *et al*, 1996). Animals that were not exposed to swim stress were given injections, bled, and perfused at comparable time points to assess any effects of these treatments on basal activity of the HPA axis. This paradigm was chosen because, in rats, chronic administration of antidepressants is required to elicit the suppression of corticosterone and reduction in c-fos expression in the PVN (Connor *et al*, 1998, 2000; Duncan *et al*, 1996).

Biochemical Analysis

Brain sections were homogenized, membranes isolated, and CB_1 receptor binding parameters determined as previously described (Hill *et al*, 2005a). For analysis of endocannabinoid content, brain regions were subjected to a lipid extraction process exactly as described previously (Patel *et al*, 2003). The content of the two primary endocannabinoids anandamide (AEA) and 2-arachidonylglycerol (2-AG) within lipid extracts was determined using isotope-dilution liquid chromatography−mass spectrometry as described previously (Patel *et al*, 2005a).

Radioimmunoassay

Blood was allowed to coagulate overnight at 4°C. The following morning, plasma was harvested by centrifugation at 12 000 r.p.m. for 20 min and was stored at −80°C until

analysis. Plasma corticosterone levels were determined by radioimmunoassay as detailed previously (Hill *et al*, 2005b).

Immunohistochemical Analysis

Fixed brains were sliced coronally into 35 μm coronal sections using a vibratome. Sections were washed in potassium phosphate-buffered saline (KPBS), incubated in a 0.4% peroxide bath, and thoroughly washed again in KPBS. Sections were then briefly exposed to 0.1% sodium borohydride solution, washed in KPBS, and incubated for 48 h at 4°C in KPBS with 2% goat serum and 0.3% Triton X-100 (loaded KPBS) containing polyclonal rabbit antisera against residues 4–17 of human fos protein (Oncogene Labs, Cambridge, MA, USA; at 1 : 26 000). Sections were subsequently washed in KPBS and incubated in biotinylated, goat anti-rabbit secondary antibody (1 : 222) for 60 min, washed again in KPBS, and transferred to a avidin–biotin complex solution (Vector Laboratories, Burlingame, CA, USA) for 60 min. Tissue was then washed in KPBS, transferred to 1.0 M sodium acetate, and developed using a diaminobenzidine reaction driven by glucose oxidase. Tissue was subsequently mounted, dehydrated, and coverslipped. Light-level images were captured using a Hamamatsu optical system coupled to a Macintosh computer running Open Lab imaging and measuring software (Quorum Technologies, Guelph, ON, Canada). Fos-ir cell counts were taken by an observer blind to animal status in regularly spaced (150-μm) intervals through the rostrocaudal extent of the paraventricular cell group. Positive cells were identified as those expressing a black nuclear reaction product. Discrete localization of Fos-ir profiles to the dorsal medial parvocellular (neuroendocrine anterior pituitary regulating) population of the PVN (mpdPVN) was accomplished by limiting the region of interest to the area medial to the magnocellular population, lateral to the periventricular zone, and ventral to the dorsal cap, as has been carried out previously. This conservative analysis ensures that all slices are examined at the same rostral–caudal axis and also ensures that the region of interest is where all the CRH neurosecretory cells, which communicate to the anterior pituitary to stimulate ACTH, are amassed (Viau *et al*, 2005; Viau and Sawchenko, 2002). Cell number estimates were generated by counting bilaterally the number of Fos-positive cells through the medial parvocellular cell population, averaged by dividing cell counts by slice number, and corrected for sampling frequency (one in five sections, 150-μm intervals) by multiplying this product by a factor of five. Furthermore, to ensure that any determinations were not artifacts of PVN area, we also performed density analysis, determining how many fos-ir cells were present per mm^2 of the mpdPVN. Results thus represent estimates of the total number of Fos-positive cells per mpdPVN region as well as number of Fos-ir cells per mm^2.

Statistics

Cannabinoid CB_1 receptor binding parameters and endocannabinoid content were analyzed by a *t*-test comparing vehicle-treated animals with desipramine-treated animals. Analysis of the stress-induced hormonal and cellular effects was performed using a univariate analysis of variance, with drug treatment and swim exposure as fixed factors. *Post hoc* tests were performed using a Tukey's HSD test. Significance was established against an alpha value of 0.05.

RESULTS

Chronic Treatment with the Tricyclic Antidepressant Desipramine Upregulates the CB_1 Receptor in Key Regions of the Stress Axis

Animals that had been treated for 21 days with desipramine exhibited significant increases in the binding site density (B_{max}) of the cannabinoid CB_1 receptor in the hippocampus (t (5) = 4. 43, $p<0.01$) and the hypothalamus (t (6) = 3.76, $p<0.01$). There was no significant effect of desipramine treatment on the B_{max} of the CB_1 receptor in the prefrontal cortex (t (6) = 2.20, $p>0.05$) or amygdala (t (5) = 0.31, $p>0.05$). Data regarding the effects of desipramine treatment on the B_{max} of the cannabinoid CB_1 receptor can be seen in Figure 1. There was no significant effect of chronic desipramine treatment upon the affinity (K_d) of [^{3}H]CP 55,940 for the CB_1 receptor in the prefrontal cortex (t (6) = −0.16, $p>0.05$; vehicle: 0.27 ± 0.04 nM *vs* desipramine: 0.28 ± 0.07 nM), the hippocampus (t (5) = 1.55, $p>0.05$; vehicle: 1.14 ± 0.14 nM *vs* desipramine 1.67 ± 0.28 nM), the hypothalamus (t (6) = 0.76, $p>0.05$; vehicle: 2.21 ± 1.24 nM *vs* desipramine: 2.87 ± 0.85 nM), or the amygdala (t (5) = 0.31, $p>0.05$; vehicle: 1.7 ± 0.86 nM *vs* desipramine: 0.82 ± 0.27 nM).

Animals that had been treated with desipramine for 21 days did not exhibit any significant changes in prefrontal cortical AEA (t (14) = 0.10, $p>0.05$) or 2-AG content (t (13) = 1.70, $p>0.05$); hippocampal AEA (t (14) = 0.52, $p>0.05$) or 2-AG content (t (13) = 0.31, $p>0.05$); hypothalamic AEA (t (11) = 0.95, $p>0.05$) or 2-AG content (t (11) = 0.15, $p>0.05$); or amygdalar AEA (t (14) = 1.11, $p>0.05$) or 2-AG content (t (14) = 0.99, $p>0.05$). Data regarding the effects of chronic desipramine treatment on endocannabinoid content in these brain structures can be seen in Table 1.

Upregulation of the Endocannabinoid System Contributes to the Suppression of Stress-Induced Activation of the HPA Axis Elicited by Chronic Desipramine Treatment

To examine whether the upregulation of the CB_1 receptor following chronic desipramine treatment plays a functional role in the effects of this treatment on HPA axis responsivity, we determined whether acute blockade of the CB_1 receptor affected swim stress-induced corticosterone release and c-fos expression in the mpdPVN. There was a significant interaction between drug treatment and exposure to swim stress on plasma corticosterone concentration (F (3, 40) = 3.14, $p<0.05$), with a significant main effect of exposure to stress (F (1, 40) = 469.26, $p<0.01$), but no main effect of drug treatment (F (3, 40) = 1.45, $p>0.05$). *Post hoc* analyses revealed that exposure to swim stress increased plasma corticosterone ($p<0.01$ for all treatment conditions); however, chronic pretreatment with desipramine resulted in a significant reduction in plasma corticosterone following stress exposure compared to saline-treated, stressed rats ($p<0.04$). Acute treatment with AM251 completely

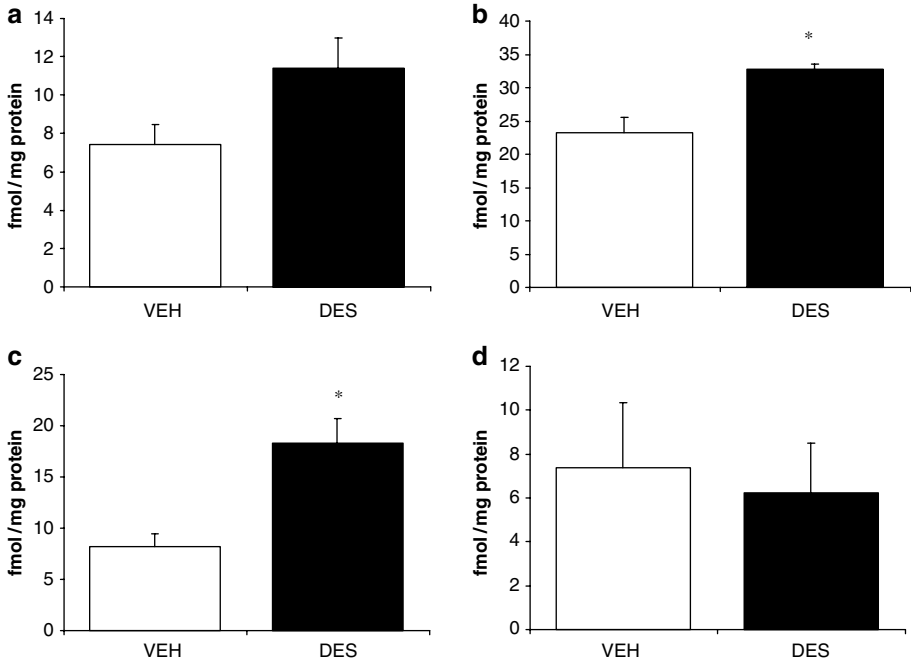

Figure 1 The effect of chronic desipramine (DES; 10 mg/kg) treatment on the maximal binding (B_{max}) of the cannabinoid CB_1 receptor as measured by [^{3}H]CP55,940 binding in the (a) prefrontal cortex, (b) hippocampus, (c) hypothalamus, and (d) amygdala, relative to vehicle (VEH)-treated rats. Data are presented as mean values ± SEM ($n = 3$–4 subjects/group). Significant differences ($p < 0.05$) are denoted by *.

Table 1 Effect of Chronic Desipramine (10 mg/kg) Treatment on Brain Regional Endocannabinoid Content

	Control	Desipramine
Prefrontal cortex		
AEA (pmol/g tissue)	9.99 ± 0.56	10.09 ± 0.85
2-AG (nmol/g tissue)	4.58 ± 0.43	5.66 ± 0.46
Hippocampus		
AEA (pmol/g tissue)	23.76 ± 0.55	23.25 ± 0.84
2-AG (nmol/g tissue)	8.27 ± 0.22	8.41 ± 0.37
Hypothalamus		
AEA (pmol/g tissue)	2.79 ± 0.26	2.42 ± 0.31
2-AG (nmol/g tissue)	7.81 ± 0.52	7.93 ± 0.71
Amygdala		
AEA (pmol/g tissue)	8.03 ± 0.76	6.82 ± 0.78
2-AG (nmol/g tissue)	7.56 ± 0.81	8.68 ± 0.79

Desipramine treatment for 21 days did not change the content of either anandamide (AEA) or 2-arachidonylglycerol (2-AG) in any brain region examined. Data are presented as mean values ± SEM ($n = 6$–8 subjects/group).

occluded the desipramine-induced reduction in plasma corticosterone ($p < 0.05$), whereas AM251 administration alone had no effect on the stress-induced increase in plasma corticosterone ($p > 0.05$). There was no effect of either desipramine or AM251 treatment on plasma corticosterone levels in animals that had not been exposed to the stressor. These data can be seen in Table 2.

Table 2 Effect of Chronic Desipramine (10 mg/kg) Treatment and Acute Pharmacological Blockade of the Cannabinoid CB_1 Receptor, Using the CB_1 Receptor Antagonist AM251 (1 mg/kg), on Plasma Corticosterone Levels Under Basal Conditions and Following Exposure to Swim Stress

	No stress	Swim stress
Plasma corticosterone (ng/ml)		
Saline–vehicle	92.0 ± 22.1	537.4 ± 19.3*
Desipramine–vehicle	138.2 ± 31.8	417.7 ± 11.9*‡
Saline–AM251	119.4 ± 45.6	541.0 ± 16.7*
Desipramine–AM251	137.2 ± 13.9	536.9 ± 32.4*

Data are presented as mean values ± SEM ($n = 5$–7 subjects/group). Significant differences between stress and no stress groups for each respective treatment ($p < 0.05$) are denoted by *; significant differences between desipramine–vehicle swim stress group and all other swim stress conditions ($p < 0.05$) are denoted by ‡.

With respect to number of fos-ir cells present in the mpdPVN, results paralleled the hormonal data. Two-way ANOVA revealed a significant interaction between exposure to stress and drug treatment ($F(3, 25) = 5.00$, $p < 0.01$), with significant main effects of both stress exposure ($F(1, 25) = 235.71$, $p < 0.01$) and drug treatment ($F(3, 25) = 8.98$, $p < 0.01$). *Post hoc* analyses demonstrated that exposure to swim stress significantly increased fos expression in the mpdPVN in all treatment groups (all p's < 0.01); however, animals that had been pretreated with desipramine exhibited significantly lower levels of c-fos expression in the mpdPVN than all other groups exposed to stress (all p's < 0.01). Acute treatment with AM251 prevented this reduction in fos-ir in the mpdPVN in desipramine-treated

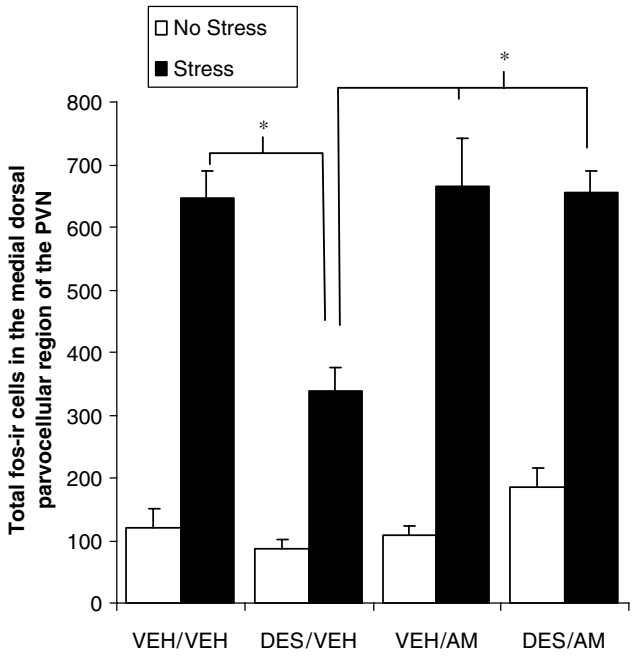

Figure 2 The effect of chronic administration of desipramine (DES; 10 mg/kg), and the influence of acute cannabinoid CB_1 receptor blockade through administration of AM251 (AM; 1 mg/kg), on both basal and stress-induced elevations in the total number of fos immunoreactive-like (fos-ir) cells in the medial dorsal parvocellular population of the paraventricular nucleus (mpdPVN) of the hypothalamus. Data are presented as mean values ± SEM ($n = 4$–5 subjects/group). Significant differences ($p < 0.05$) are denoted by *.

animals ($p < 0.01$). These data can be seen in Figure 2, and photomicrographs illustrating the changes in c-fos expression in the mpdPVN can be seen in Figure 3.

A comparable trend was seen in density measurements of fos-ir cells per mm^2 of the mpdPVN. Two-way ANOVA of the c-fos density measurements revealed a significant interaction between exposure to stress and drug treatment ($F(3, 25) = 7.36$, $p < 0.01$; data not shown), with significant main effects of both stress exposure ($F(1, 25) = 316.01$, $p < 0.01$) and drug treatment ($F(3, 25) = 14.30$, $p < 0.01$). As with total fos-ir cells in the mpdPVN, the density of fos-ir cells showed that all animals exposed to the swim stress exhibited a significant increase in fos-ir (all p's < 0.01); however, those that had been pretreated with desipramine exhibited a significantly lower density of fos-ir cells ($p < 0.01$). The reduction in the density of fos-ir cells was elicited by chronic desipramine treatment in rats treated with AM251 ($p < 0.01$).

DISCUSSION

This study provides the first demonstration to date that chronic treatment with the tricyclic antidepressant desipramine produces an increase in the density of CB_1 cannabinoid receptor binding sites in the hippocampus and hypothalamus without effects on either the binding affinity of the agonist [^{3}H]CP 55,940 or tissue contents of the two major endocannabinoids in any brain structure examined. As both the hippocampus and hypothalamus are involved in processing and regulating responses to stress, these data suggest that chronic desipramine treatment could change stress responsivity as a result of changes in CB_1 receptor signaling in these brain regions. These findings are intriguing given that chronic unpredictable stress, an animal model of depression, results in a significant reduction in CB_1 receptor binding site density and a down-regulation of the endocannabinoid 2-AG in the hippocampus (Hill *et al*, 2005a). This bidirectional regulation of hippocampal CB_1 receptors by stress and antidepressants suggests that CB1 receptor/endocannabinoid signaling in the hippocampus could be relevant for the development and treatment of depression. Interestingly, long-term desipramine treatment did not affect endocannabinoid content in any brain structure examined, although brain regional endocannabinoid content is sensitive to stress exposure (Hill *et al*, 2005a; Patel *et al*, 2004, 2005b) and is affected by acute manipulation of monoamine receptor activity (Patel *et al*, 2003; Giuffrida *et al*, 1999).

The mechanism by which chronic tricyclic antidepressant treatment regulates CB_1 receptor expression is currently unknown; however, previous studies have demonstrated that antidepressant treatment can increase receptor trafficking and upregulate membrane expression of receptors, such as the AMPA receptor (Martinez-Turrillas *et al*, 2002). The CB_1 receptor is known to exist at both the membrane level and in intracellular endosomic stores, with the vast majority (~85%) of the receptor population typically existing in intracellular vesicles (Leterrier *et al*, 2004). Thus, the increase in CB_1 receptor binding sites following tricyclic antidepressant treatment may be owing to an increase in receptor trafficking such that a higher proportion of CB_1 receptors are active at the membrane site. This increase in active expression of the CB_1 receptor may be an adaptive response elicited by treatment with desipramine. The primary pharmacological property of desipramine is its ability to inhibit norepinephrine reuptake and thus potentiate the synaptic action of norepinephrine (Frazer, 1997; Wong *et al*, 2000). Both *in vivo* and *ex vivo* work in rodent and human tissue has demonstrated that CB_1 receptors in the hippocampus and hypothalamus negatively regulate noradrenergic neurotransmission (Tzavara *et al*, 2001; Schlicker *et al*, 1997). Thus, the upregulation of CB_1 receptors in the hippocampus and hypothalamus seen in this study could be an adaptive response launched by the central nervous system to decrease noradrenergic transmission by increasing the density of presynaptic CB_1 receptors, which in turn would reduce NE release and normalize the increased synaptic availability induced by desipramine treatment.

One common functional response to chronic antidepressant treatment, especially tricyclic antidepressants, is an attenuation of stress-induced activation of the HPA axis (Duncan *et al*, 1996; Connor *et al*, 2000; de Medeiros *et al*, 2005; Butterweck *et al*, 2001). To explore the functional relevance of the changes in the endocannabinoid system induced by chronic desipramine treatment, we examined the effects of acute blockade of the CB_1 receptor with AM251 on the peak hormonal and cellular responses to stress following this antidepressant regimen. Chronic treatment with desipramine produced a significant reduction in both peak stress-induced increases in neuronal activation

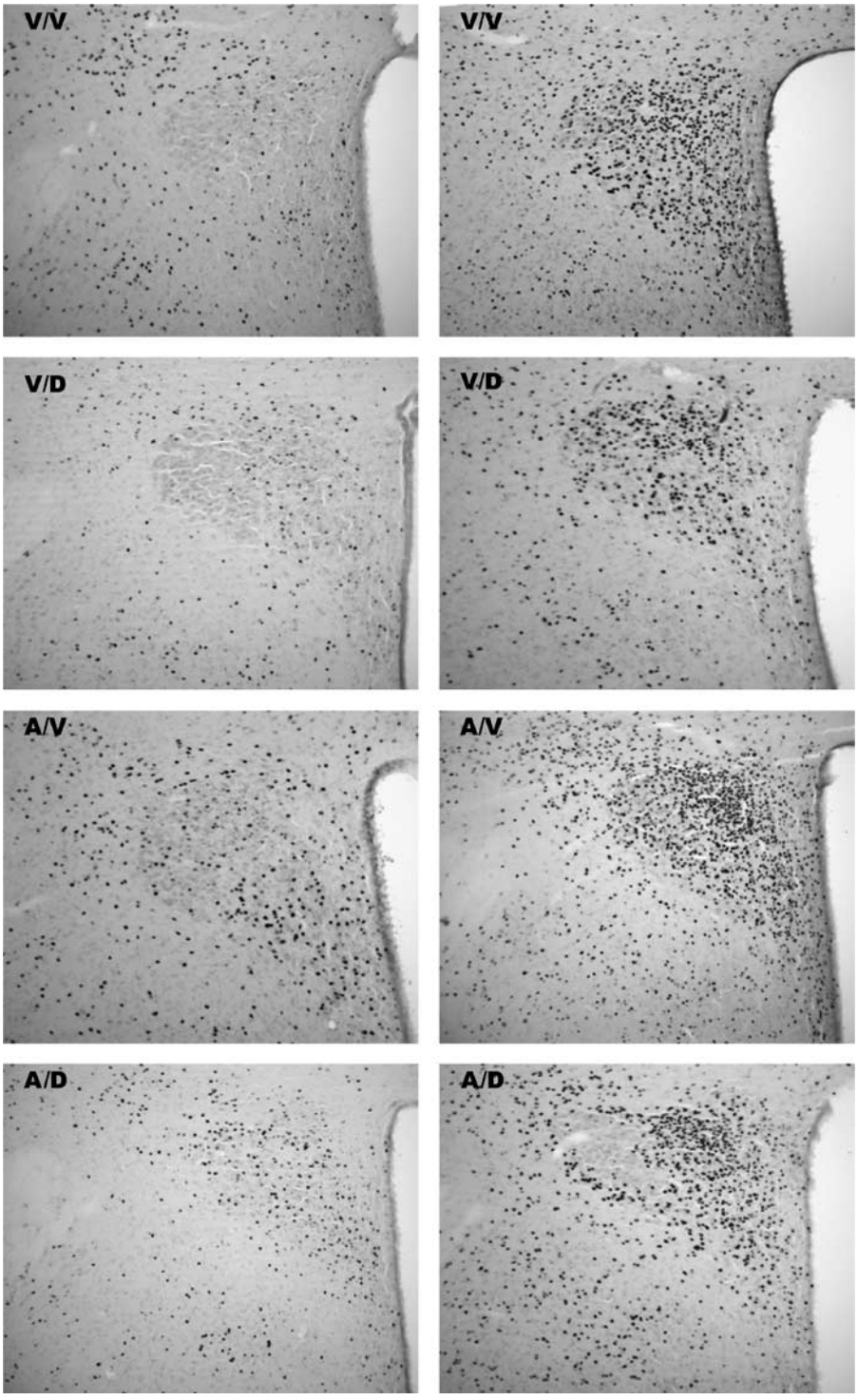

Figure 3 Representative photomicrographs of fos immunoreactivity in the mpdPVN under both basal conditions (left panel) and in response to swim stress exposure (right panel) (V = vehicle; D = desipramine; A = AM251).

within the mpdPVN and peak stress-induced increases in plasma corticosterone concentrations, as has been shown previously (Duncan *et al*, 1996; Connor *et al*, 2000). Acute treatment with AM251 completely occluded the effect of desipramine to reduce activation of the HPA axis. These data suggest that engagement of the endocannabinoid system is necessary for tricyclic antidepressants to suppress stress-induced activation of the HPA axis. However, we cannot rule out changes in the time course of the activation of the HPA axis; for example, AM251 treatment could delay the response or, alternatively, enhance its recovery. Future studies will examine these possibilities.

This interaction between antidepressants and the endocannabinoid system could occur at the level of the hypothalamus, a region in which desipramine increased CB_1 receptor binding. Recent data have demonstrated that

CB_1 receptors in the PVN of the hypothalamus gate glutamatergic fibers, which activate the HPA axis (Di *et al*, 2003), which suggests that the endocannabinoid system can regulate glutamate-induced activation of the HPA axis. This hypothesis has received *in vivo* support from experiments demonstrating that genetic deletion of the CB_1 receptor exacerbates stress-induced activation of the HPA axis, whereas enhancement of endocannabinoid signaling can attenuate stress-induced activation (Patel *et al*, 2004; Barna *et al*, 2004). Thus, the upregulation of the CB_1 receptor in the hypothalamus seen in this study is consistent with suppression of stress-induced activation of the HPA axis following desipramine treatment. The suppression of corticosterone secretion following exposure to stress was accompanied by a reduction in neuronal activation of the mpdPVN of the hypothalamus, an area that contains all the CRH neurosecretory cells that regulate ACTH secretion through communication with the anterior pituitary. Although we did not assess CRH mRNA in this study, the region of interest we analyzed in the mpdPVN here is where all CRH neurosecretory cells are amassed (Viau and Sawchenko, 2002; Viau *et al*, 2005) suggesting that a suppression of stress-induced activation of CRH neurosecretory cells is likely mediating the effect of desipramine in this study. In support of this hypothesis, a recent report has demonstrated that reductions in stress-induced CRH transcription are associated with suppression of stress-induced peripheral corticosterone secretion following desipramine treatment (Conti *et al*, 2004). As such, the current data suggest that the ability of desipramine to suppress HPA axis activation could be through an upregulation of CB_1 receptors in the mpdPVN, which regulate excitatory input to CRH neurosecretory cells, which in turn may lead to an increased suppression of stress-induced corticosterone secretion. However, given that CB_1 receptors were also increased in the hippocampus, and the hippocampus is known to exert a potent role in regulation and feedback of the HPA axis (Jacobson and Sapolsky, 1991; Herman *et al*, 1998; Mueller *et al*, 2004), the possibility does exist that changes in the endocannabinoid system upstream of the mpdPVN could elicit a net reduction in activation of incoming afferents to the neurosecretory cells of the mpdPVN. Regardless of the locus of action, the current data suggest that the endocannabinoid system is involved in the effects of chronic desipramine administration to reduce HPA axis activation by stress.

These data also support our recently proposed hypothesis that the endocannabinoid system acts as a buffer against the effects of stress in the brain (Patel *et al*, 2005b). Specifically, repeated episodes of homotypic stress results in a habituation of the stress response that is accompanied by an increase in the tissue contents of endocannabinoid ligands in the limbic system and the hypothalamus, and acute treatment with a CB_1 receptor antagonist can reverse habituation to repeated homotypic stress (Patel *et al*, 2004, 2005b). These data suggest that the endocannabinoid system acts to modulate or dampen activation of the neural stress axis (Patel *et al*, 2005b). We now extend this hypothesis to include that chronic exposure to desipramine (and perhaps other antidepressant drugs and therapies) also upregulates the endocannabinoid system, which, in turn, dampens the stress axis in a manner similar to habituation. This hypothesis is supported by recent clinical data demonstrating that plasma endocannabinoid content is increased in minor depression, but decreased in major depression, suggesting that successful upregulation of the endocannabinoid system can prevent the progression of stress into affective disease (Miller *et al*, 2005). Preclinical animal data also support this contention as transgenic mice that lack the CB_1 receptor exhibit an increased susceptibility to the anhedonic effects of chronic stress, suggesting that this system may be integral to the development and maintenance of effective coping strategies to stress (Martin *et al*, 2002).

Given that the ability of antidepressants to regulate the HPA axis is tightly coupled to their clinical efficacy (Holsboer and Barden, 1996; Greden *et al*, 1983; Ribeiro *et al*, 1993; Zobel *et al*, 2001), these data suggest that upregulation of the endocannabinoid system is involved in the normalization of hypercortisolemia that accompanies remission of depression. These data also support the suggestion that the endocannabinoid system could serve as a suitable target for the development of novel antidepressants (Hill and Gorzalka, 2005a; Jiang *et al*, 2005; Gobbi *et al*, 2005), especially for melancholic depression (Hill and Gorzalka, 2005b), which exhibits a preferential response to tricyclic antidepressants and reliably exhibits hyperactivity of the HPA axis (Rush and Weissenburger, 1994; Bielski and Friedel, 1976; Gold and Chrousos, 2002).

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