Published in final edited form as:

Neurosci Res. 2008 September ; 62(1): 62–65. doi:10.1016/j.neures.2008.05.009.

Amphetamine Treatment Increases Corticotropin-Releasing Factor Receptors in the Dorsal Raphe Nucleus

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Abstract

Psychostimulant use increases anxious behavior, likely through interactions between central corticotropin-releasing factor (CRF) and serotonergic systems. The current study examined whether chronic amphetamine treatment (2.5 mg/kg, 14 days) or withdrawal altered CRF receptor densities in the serotonergic dorsal raphe nucleus (dRN). Amphetamine treatment increased CRF₂ receptor densities in most subregions of the dRN, and CRF₂ receptors were still elevated following 6 weeks of withdrawal. No changes in CRF₁ receptor densities were observed following amphetamine treatment or during withdrawal. Selective increases in dRN CRF₂ receptors may be related to increased anxiety-like behaviors following psychostimulant use.

Keywords

Serotonin; Anxiety; Rat; CRF; Dorsal Raphe Nucleus; Amphetamine

The cycle of addiction is thought to be maintained by negative affective symptoms that are manifest upon cessation of drug taking (Koob, 2003). In rats, withdrawal from chronic psychostimulant administration increases anxiety-like and depressive behaviors (Sarnyai et al., 1995; Perrine et al., 2008). Corticotropin-releasing factor (CRF) plays an important role in mediating anxiety behavior (Bale, 2005; Shepard and Meyers, 2008). Activation of CRF receptors can increase serotonergic neuronal activity in the dorsal raphe nucleus (dRN) (Lowry et al., 2000; Pernar et al., 2004) and increases serotonin release in forebrain limbic regions important for anxiety-like behaviors (Amat et al., 2004; Forster et al., 2006; Lukkes et al., 2008). Stress-induced alterations of serotonin release within forebrain limbic regions are suppressed by central CRF receptor antagonism (Price et al., 2002; Mo et al., 2008). Furthermore, intracerebroventricular administration of CRF antiserum reduces anxiety-like behavior of cocaine-treated rats in withdrawal (Sarnyai et al., 1995). Given the relationship between CRF, serotonin activity and anxiety-like behaviors, psychostimulant-induced changes to CRF receptor levels in the dRN could represent an important pathological alteration related to negative affect following psychostimulant use.

Adult male Sprague Dawley rats (n = 24; University of South Dakota Animal Resource Center) were injected with amphetamine (2.5 mg/kg, ip.) or saline daily for 14 days. The amphetamine

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dose and injection schedule were based on preliminary studies showing behavioral sensitization and increased anxiety-like behavior following treatment at 2 weeks withdrawal (Forster et al., 2007). Twenty hours or 6 weeks following the last injection, rats were anesthetized with pentobarbital (100 mg/kg, ip.) and transcardially perfused with 0.1 M phosphate-buffered saline followed by 4% paraformaldehyde (n = 6 per treatment group at each time point).

Immunocytochemistry provides spatial resolution/sensitivity, allowing CRF receptor densities within dRN subregions to be analyzed separately (Figure 1). Sections of the dRN (30 μ m) from saline and amphetamine treated rats were processed together, and consecutive serial sections were processed separately for CRF₁ and CRF₂ receptors, since both receptors are observed in the dRN (Day et al., 2004). Sections were incubated in 10% normal rabbit serum (Jackson ImmunoResearch, West Grove, PA) for 2 hours prior to either goat CRF₁ polyclonal antibody (1:200; Santa Cruz Biotech, #SC-12381) or goat CRF₂ polyclonal antibody (1:300; Santa Cruz Biotech, #SC-1826) for 20 hours at 4°C. Specificity of the CRF₁ and CRF₂ antibodies in the dRN were determined using purified CRF₁ and CRF₂ blocking peptides (1:3 ratio of primary antibody to blocking peptide; Santa Cruz Biotech, #SC-12381P and #SC-1826P, respectively). After rinsing, sections were incubated for 2 hours at room temperature in Cy2-conjugated rabbit anti-goat secondary antibody (1:200; Jackson ImmunoResearch #305-225-003). Sections were visualized using a fluorescence microscope (Zeiss Axioskop 2-Mot, Thornwood, NY). These procedures were approved by the Institutional Animal Care and Use Committee of the University of South Dakota.

The average luminosity of the pixels within each dRN subregion was obtained as a measure of receptor density by experimenters blind to treatment, and subtracted from background values obtained from regions where no cellular staining was observed to normalize for background staining (Lindahl and Keifer, 2004). Receptor densities (Figures 2 and 3) and the ratio of CRF₂/CRF₁ receptors (Table 1) within each subregion of the dRN and were compared between saline and amphetamine treated rats using separate one-way ANOVA (SigmaStat v.2.03).

As previously described (Day et al., 2004), CRF₁ and CRF₂ receptors were located throughout the dRN. Staining was predominantly localized to soma but some punctate staining was also apparent (Figure 1C). There were no significant differences between amphetamine and saline treated rats in normalized CRF₁ receptor densities within the anterior medial ($F_{1,10}=2.47$, $p=0.177$), anterior lateral ($F_{1,10}=4.946$, $p=0.077$) or posterior ($F_{1,10}=1.524$, $p=0.272$) subregions of the dRN when measured 20 hours following last injection (Figure 2A). Likewise, there were no significant differences in CRF₁ receptor densities within the anterior lateral ($F_{1,10}=0.578$, $p=0.489$), anterior medial ($F_{1,10}=1.259$, $p=0.325$) or posterior ($F_{1,10}=1.556$, $p=0.280$) subregions of the dRN following 6 weeks of withdrawal (Figure 2B). In contrast, normalized CRF₂ receptor densities were significantly higher in amphetamine treated rats within the anterior lateral ($F_{1,10}=73.706$, $p=0.001$) and anterior medial ($F_{1,10}=91.636$, $p<0.001$), but not the posterior ($F_{1,10}=0.981$, $p=0.367$) subregions of the dRN 20 hours following the last injection (Figure 3A). Similarly, the ratio of CRF₂/CRF₁ receptor densities of amphetamine pretreated rats was significantly higher than saline pretreated rats in the anterior ($p<0.05$) but not posterior ($p=0.203$) subregions of the dRN, 20 hours following last treatment (Table 1). Following 6 weeks of withdrawal, CRF₂ receptor densities were significantly higher in amphetamine treated rats within the anterior lateral ($F_{1,10}=12.130$, $p=0.018$), anterior medial ($F_{1,10}=13.163$, $p=0.015$) and posterior ($F_{1,10}=16.481$, $p=0.010$) subregions of the dRN (Figure 3B). The ratio of CRF₂/CRF₁ receptor densities of amphetamine treated rats were also significantly increased within these same dRN subregions at 6 weeks withdrawal ($p<0.05$; Table 1).

Overall, amphetamine treatment (2.5 mg/kg) for 14 days increased CRF₂ receptor densities within the anterior dRN, which remained elevated following 6 weeks of withdrawal. Since

acute administration of amphetamine activates CRF neurons (Rotllant et al., 2007), our results suggest that repeated stimulation of CRF neurons by amphetamine results in long-lasting up-regulation of CRF₂ receptors in the anterior dRN. Furthermore, the withdrawal process resulted in elevated CRF₂ receptor densities in the posterior dRN that were not initially observed following amphetamine treatment. The dRN subregions in which CRF₂ receptor levels were elevated during amphetamine withdrawal project to many limbic brain regions that mediate stress and anxiety, such as the amygdala, hippocampus and nucleus accumbens (reviewed by Lowry, et al., 2000). Activation of CRF₂ receptors within the dRN increases serotonin release in these limbic regions (Amat et al., 2004; Lukkes et al., 2008). Therefore, a functional consequence of increased dRN CRF₂ receptor levels during amphetamine withdrawal could be enhanced serotonergic activity within the limbic system. Also, upon further administration of the drug, psychostimulant-induced activation of CRF systems could result in enhanced CRF₂ receptor activation. This may contribute to the sensitized profile of psychostimulant-treated rats, since CRF₂ receptors increase serotonin release in the nucleus accumbens (Lukkes et al., 2008), and serotonin enhances accumbal dopamine release (Parsons and Justice, 1993).

Our results suggest that psychostimulant-induced depressive and anxiety-like behavior may result from increased CRF₂ receptor activity in the dRN. Mice globally deficient in the CRF₂ receptor show increased anxiety-like behavior, implying an anxiolytic role for CRF₂ receptors (Bale, 2005). However, studies specifically targeting the dRN suggest that CRF₂ receptors mediate the adverse behavioral consequences of chronic stress. For example, CRF₂ receptor antagonism in the posterior dRN prevents learned helplessness behavior (Hammack et al., 2003). On the basis of the current results, future directions should examine whether dRN CRF₂ receptors in the dRN represent an important therapeutic target for the treatment of psychostimulant induced depressive and anxiety-like behaviors during withdrawal.

Acknowledgements

Supported by NIH COBRE P20 RR15567 and NIDA R01 DA019921. We thank Dr. Joyce Keifer, Dr. Max Mokin, Dr. Michael Watt, Jeffrey Barr, and Andrew Burke for their valuable assistance with these experiments.

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Figure 1.

(A) Anterior and (B) posterior subregions of the rat dRN analyzed for CRF₁ and CRF₂ receptor densities. Lateral regions were not analyzed in posterior sections since the dRN is a medial structure at this level (Paxinos and Watson, 1997). Figures were adapted from Paxinos and Watson (1997). (C) Representative photomicrographs (40 × magnification) of CRF₁ and CRF₂ receptor immunofluorescence in the anterior medial dRN of the rat. White bars = 10 μm.

Figure 2.

Normalized CRF₁ receptor densities within the dRN did not differ between amphetamine and saline treated rats (**A**) 20 hours or (**B**) 6 weeks following the last injection.

Figure 3.

Normalized CRF₂ receptor densities within subregions of the dRN were increased in amphetamine treated rats compared to saline controls (**A**) 20 hours and (**B**) 6 weeks following the last injection. * $p < 0.05$

Table 1 Ratio of CRF₂/CRF₁ receptors densities in subregions of the dorsal raphe nucleus.

Region	Withdrawal Period			
	20 hours		6 weeks	
	Amphetamine	Saline	Amphetamine	Saline
Anterior Lateral	1.90+/-0.34 [*]	0.91+/-0.21	1.83+/- 0.15 [*]	0.74+/-0.18
Anterior Medial	1.90+/-0.21 [*]	0.85+/-0.11	1.81+/-0.28 [*]	0.70+/-0.14
Posterior	1.27+/-0.10	1.03+/-0.16	2.02+/-0.40 [*]	0.91+/-0.15

Data represent mean ratio +/- SEM.

* p < 0.05 compared to saline pretreated rats within the same withdrawal period