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Modulation of a 40-kDa catecholamine-regulated protein following D-amphetamine treatment in discrete brain regions

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Abstract

A 40-kDa catecholamine-regulated protein (CRP40) has been demonstrated to be expressed in the central nervous system, and is known to bind to dopamine and related catecholamines. Recently, it has been shown that dopamine D1 receptor antagonist and dopamine D2 receptor antagonist differentially modulated the CRP40 protein in the striatum. In the present study, we examined the effects of the indirect psychostimulant, D-amphetamine, on (CRP40) expression in discrete brain regions. The technique of Western immunoblotting was utilized for quantitation of CRP40 in different experimental paradigms following D-amphetamine treatment. Acute treatment with D-amphetamine (5.0 mg/kg, i.p.) caused no significant change in CRP40 levels in either of the two brain regions studied: striatum and nucleus accumbens. Chronic D-amphetamine administration (2.5 mg/kg, i.p.) significantly increased CRP40 levels in striatum and nucleus accumbens ($37.64 \pm 14.57\%$ and $27.86 \pm 8.40\%$, respectively, $P \leq 0.05$). Chronic and possibly sensitized D-amphetamine challenged rats (0.5 mg/kg, i.p.) showed a significant increase in CRP40 levels in the nucleus accumbens only ($40.49 \pm 15.91\%$, $P \leq 0.05$). Although CRP40 has a consensus motif with the 70-kDa heat shock protein (HSP70), levels of HSP70 remained unchanged under identical experimental conditions. The results of this study demonstrate selective modulation of CRP40 by D-amphetamine treatment, without affecting the 70-kDa heat shock protein.

Keywords: Catecholamine-regulated protein; D-Amphetamine; Desensitization, behavioural sensitization; Dopamine; Heat shock protein; Molecular chaperone

1. Introduction

Dopamine, a catecholamine neurotransmitter, is found in the brain and the peripheral nervous system. In the central nervous system, dopamine is involved in various functions including locomotion, cognition, and endocrine regulation (Bear et al., 2001; Kandel et al., 2000). Abnormal dopamine neurotransmission has been implicated in many central nervous system diseases, such as schizophrenia and Parkinson's disease (Lewis and Lieberman, 2000; Marcotte et al., 2001). Oxidative stress, involving excess dopaminergic activity, has been implicated in causing tissue injury and resulting in neurotoxicity (Bowyer, 2000). As well, the oxidation of dopamine and its quinone metabolites have been shown to result in free radical production (Cadet and Brannock, 1998; Halliwell et al., 1992; Jones et al., 2000; Nair and Mishra, 2001; Stokes et al., 1999). In response to heat or oxidative stress, all organisms produce antioxidants to neutralize these reactive oxygen species, allowing the organism to maintain homeostatic conditions within the brain. In addition, almost all organisms synthesize a certain set of proteins known as heat shock proteins (Ohtsuka and Suzuki, 2000; Suzuki et al., 1999). Under stress, protein structures become perturbed and denatured. Chaperone-type proteins are then expressed, which bind to damaged proteins and help repair their damaged structure. Furthermore, molecular chaperones may be implicated in synaptic plasticity, such as in long-term potentiation and the process of memory (Ohtsuka and Suzuki, 2000).

Earlier studies have shown that dopamine dysfunction can occur with the administration of dopamine receptor agonists and antagonists. Dopamine receptors are divided into two main subtypes: dopamine D1 receptors and dopamine D2 receptors (Jackson and Westlind-Danielsson, 1994; Vallone et al., 2000). Dopamine D1 and D5 receptors are known to stimulate adenylyl cyclase, while dopamine D2, D3, and D4 receptors inhibit adenylyl cyclase (Vallone et al., 2000).

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D-Amphetamine, a psychostimulant drug, acts as an indirect dopamine receptor agonist, which produces the following effects on the dopamine system: (1) promotes dopamine release and (2) blocks the dopamine transporter (Heikkila et al., 1975; Kuczenski, 1986; Kuczenski et al., 1983; Moore and Von Voigtlander, 1971; Rutledge, 1970; Von Voigtlander and Moore, 1973). Neurochemical changes following D-amphetamine treatments are poorly understood; however, it is believed that repeated intermittent administration of D-amphetamine causes long-lasting changes in neural systems, such as in the mesolimbic dopamine system (Robinson and Becker, 1986).

We have previously reported the presence of a unique class of brain-specific proteins that bind to dopamine and structurally related catecholamines. These proteins have been termed catecholamine-regulated proteins (CRPs) (Ross et al., 1993, 1995). Three species of CRP (with molecular weight of 47, 40, and 26 kDa, respectively) have been isolated. Pharmacological and biochemical characteristics have shown that there is no similarity between these proteins and known catecholamine binding proteins or receptors present in the brain (Goto et al., 2001; Modi et al., 1996; Ross et al., 1993, 1995; Sharan et al., 2001). Through molecular cloning, detailed characterization, and amino acid analysis, it has been established that the 40-kDa catecholamine-regulated protein (CRP40) belongs to a family of 70-kDa heat shock proteins. This suggests that CRP40 may serve as a molecular chaperone in the brain (Nair and Mishra, 2001).

Recent studies in our laboratory have shown that dopamine D2 receptor antagonist, haloperidol, up-regulates CRP40 expression, while the dopamine D1 receptor antagonist *R*(+)-7-chloro-8-hydroxy-3 methyl-phenyl-2,3,4,5,-tetrahydro-1-*H*-3-benzazepine (SCH 23390) significantly decreased the expression of the CRP40 protein (Sharan et al., 2001).

The present study was undertaken in order to understand the differential effects of D-amphetamine on CRP40 expression in the striatum and nucleus accumbens regions of the rat brain. Our results clearly demonstrate a differential expression of CRP40 in various experimental paradigms following D-amphetamine treatment.

2. Methods and materials

2.1. Animals and materials

Male Sprague–Dawley rats (250–300 g) were purchased from Charles River Canada (St. Constant, PQ). Animals were housed in pairs at the central animal facility located at McMaster University. The rats were kept in a room at constant temperature and humidity, under a 12-h lights on/off schedule. The rats were allowed 1 week to adjust and were given unlimited food and water. Body weights were taken on a daily basis prior to and during the drug treatment. Prior to decapitation, the rats were mildly anaesthetized with methoxyflurane, in order to reduce further suffering according to the guidelines approved by the Canadian Council for Animal Care. D-Amphetamine was purchased from Sigma, USA, under the license for controlled drugs, approved by Health and Welfare Canada.

2.2. Acute treatment of rats with D-amphetamine

For acute treatment studies, D-amphetamine groups received 5.0 mg/kg, i.p. ($n=6$) and control groups received 0.9% saline ($n=6$). The experimental design involved three time frames in order to distinguish at what point the 40-kDa catecholamine-regulated protein was differentially expressed. Group A rats were injected 5.0 mg/kg, i.p., D-amphetamine ($n=6$) and 0.9% saline ($n=6$) 4 h prior to decapitation. In addition, group B and group C were given the same doses of D-amphetamine and 0.9% saline, but were decapitated 8 and 16 h post injection, respectively. Following decapitation, the striatum and nucleus accumbens were immediately dissected out, homogenized and stored at −70 °C until Western immunoblotting was performed.

2.3. Chronic treatment of rats with D-amphetamine

For chronic treatment studies, D-amphetamine-treated groups received 2.5 mg/kg, i.p., and control groups, 0.9% saline, which was based on dose regimen from past studies on animal models of amphetamine psychosis that induce rats to enter into a robust behavioural sensitization (Kuczenski et al., 1997). The rats were divided into four groups designated as A–D, in order to distinguish at what point the 40-kDa catecholamine-regulated protein was expressed. Group A rats were injected intermittently for 5 days, at the same time each day, 2.5 mg/kg, i.p., D-amphetamine ($n=6$), 0.9% saline ($n=6$) and sacrificed on the 6th day. Group B rats were injected intermittently for 5 days, at the same time each day, 2.5 mg/kg, i.p., D-amphetamine ($n=6$), 0.9% saline ($n=6$) and sacrificed 14 days later. Group C rats were injected intermittently for 5 days, 2.5 mg/kg, i.p., D-amphetamine, 0.9% saline ($n=6$), left untreated for 14 days, then challenged with 0.5 mg/kg, i.p., D-amphetamine. Both control and D-amphetamine-treated rats were sacrificed 4 h later. Group D rats followed the same procedure as group C, except decapitation occurred 8 h following the challenge dose. Following decapitation, the striatum and nucleus accumbens were dissected out, homogenized, and stored at −70 °C until Western immunoblotting was performed.

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The tissues were homogenized in a Tris buffer (50 mM Tris, 1 mM EDTA at pH 7.4). Protein concentration was determined by the Bradford method (Bradford, 1976). Before the SDS-PAGE, a gel loading buffer (0.625 M Tris, 10% glycerol, 2% sodium dodecyl sulphate (SDS), 0.05% β-mercaptoethanol, and 0.01% bromphenol blue, pH 6.8)

was prepared and added to 20 μl of protein homogenate. The mixture was put in microtubes and boiled for 4 min to denature the proteins. The proteins were separated by SDS-PAGE electrophoresis using 12% acrylamide (Laemmli, 1970) and immersed in a running buffer (0.025 M Tris, pH 8.3, 0.2 M glycine, 0.1% SDS) at 65–100 V for 2 h.

2.5. Western immunoblotting

The separated proteins were transferred onto nitrocellulose electrophoretically, in a Towbin transfer buffer (0.012 M Tris, 0.096 M glycine, 10% methanol, pH 8.5) for 1.0 h at 100 V. The nitrocellulose paper was then put in a low-affinity nonspecific protein blocking agent (5% skim milk, Tris-buffered saline (TBS), 0.05 M Tris, 0.15 M NaCl, pH 8.5, 0.2% Tween-20 (T-20)) for 1 h at room temperature. The nitrocellulose membrane was then incubated with rabbit anti-CRP40 antibody (1:10,000 dilution) overnight at 4 °C and washed three times with TBS/T-20 buffer. The nitrocellulose was then incubated with a secondary antibody (horseradish peroxidase-conjugated donkey anti-rabbit antibodies, 1:5000 dilution) for 1 h at room temperature, and the blots were developed by chemiluminescence method and exposed to Kodak X-OMAT film. Quantification of CRP40 protein was carried out using a computerized image analysis system (Northern exposure, Empix Imaging).

2.6. Accuracy of gel loading and transfer of proteins

The accuracy of gel loading was determined by (a) establishing linearity of protein concentration, (b) running samples in alternate lanes, and (c) performing Ponceau-S staining of protein bands.

2.7. Statistical analysis

The data are presented graphically as relative optical density ± standard error of the mean (S.E.M.). The data were normalized for all lanes to give a more objective approach. In order to make calculations, the optical density of the control group was set to 100. A Student's *t*-test was then used to compare the optical density of the control and amphetamine-treated rats. Results were considered significant at $P \leq 0.05$. Statistical analysis was performed between groups, using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc comparison test using Graphpad Prism software, San Diego, CA.

3. Results

3.1. Acute D-amphetamine administration does not affect CRP40 expression in the striatum and nucleus accumbens

In order to study the differential effects of an indirect agonist on the levels of CRP40, animals were treated with the psychostimulant D-amphetamine and levels of CRP40 in the striatum and nucleus accumbens were measured by Western immunoblotting. The results demonstrate that there is no effect of acute D-amphetamine treatment (5.0 mg/kg, i.p.) on CRP40 expression in any of the brain regions, at any of the three time frames studied (4, 8, and 16 h). These results are shown in Fig. 1.

3.2. Chronic D-amphetamine administration, group A rats demonstrated an increase in CRP40 levels in both the striatum and nucleus accumbens

The results shown in Fig. 2A and B demonstrate that CRP40 levels significantly increased following chronic treatment of D-amphetamine (2.5 mg/kg, i.p., 5 days) in the striatum and nucleus accumbens (37.64 ± 14.57% and 27.86 ± 8.40%, respectively, $P \leq 0.05$). This dose regimen was used because it has been shown to cause robust behavioural sensitization in animal models of amphetamine psychosis (Kuczenski et al., 1997; Robinson and Becker, 1986). The intermittent treatment of D-amphetamine in rats results in neural alterations in both the striatum and nucleus accumbens.

3.3. Chronic D-amphetamine administration, group B rats demonstrated no change in CRP40 expression in the striatum and nucleus accumbens

Group B rats were injected with 2.5 mg/kg, i.p., D-amphetamine for 5 consecutive days and left untreated for 14 days and decapitation occurred on the 19th day. Analysis shown in Fig. 3 indicates no alteration of CRP40 levels, following D-amphetamine treatment in the striatum and nucleus accumbens. This suggests that the 14-day withdrawal period was enough time to allow CRP40 levels to return to normal levels.

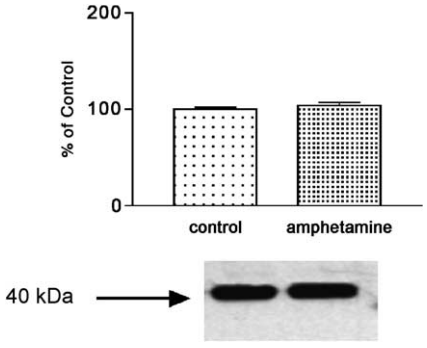

Fig. 1. Effects of acute (8 h) D-amphetamine treatment (5.0 mg/kg i.p.) on CRP40 levels in the rat striatum. Data are presented as mean ± S.E.M. ($n = 6$). Statistical significance is expressed as $P \leq 0.05$. A representative immunoblot is shown below the bar graph.

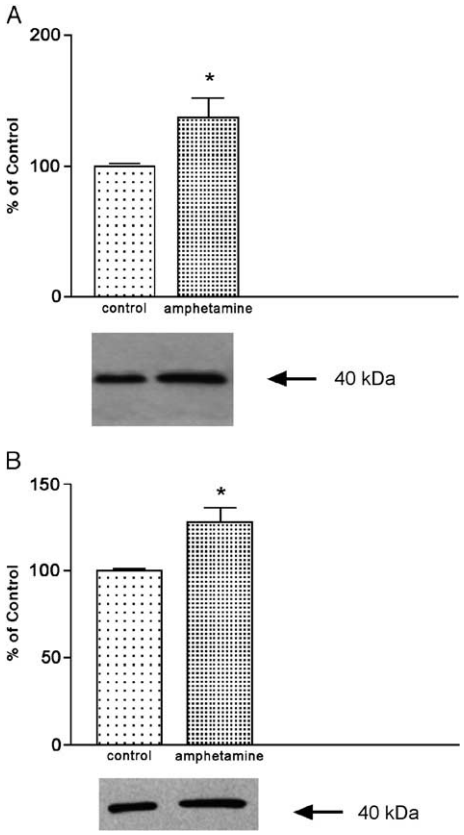

Fig. 2. (A) Effects of chronic D-amphetamine treatment (5 days, 2.5 mg/kg i.p.) on CRP40 levels in rat striatum. Data are presented as mean ± S.E.M. ($n=6$). Statistical significance is expressed as $*P \leq 0.05$. A representative immunoblot is shown below the bar graph. (B) Effects of chronic D-amphetamine treatment (5 days, 2.5 mg/kg i.p.) on CRP40 levels in rat nucleus accumbens. Data are presented as mean ± S.E.M. ($n=6$). Statistical significance is expressed as $*P \leq 0.05$. A representative immunoblot is shown below the bar graph.

3.4. Chronic D-amphetamine administration, group C rats demonstrated no change in CRP40 expression in both striatum and nucleus accumbens

Group C rats were injected with 2.5 mg/kg, i.p., D-amphetamine for 5 consecutive days, left untreated for 14 days and then challenged once with 0.5 mg/kg, i.p., D-amphetamine to both the control and drug-treated rats and decapitated 4 h later. Western immunoblot analysis demonstrated no change in CRP40 levels in both the striatum and nucleus accumbens. This suggests that the challenge dose was not large enough to cause a response of CRP40 levels, or more time was needed for observation of CRP40 expression. These results are summarized in Fig. 4.

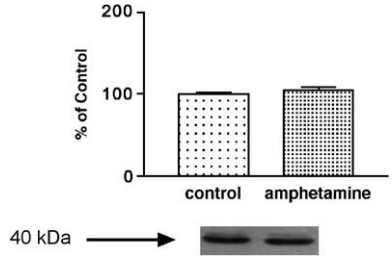

Fig. 3. Effects of chronic D-amphetamine treatment, group B (5 days, 14-day withdrawal, 2.5 mg/kg i.p.) in the rat striatum. Data are presented as mean ± S.E.M. ($n=6$). Statistical significance is expressed as $P \leq 0.05$. A representative immunoblot is shown below the bar graph.

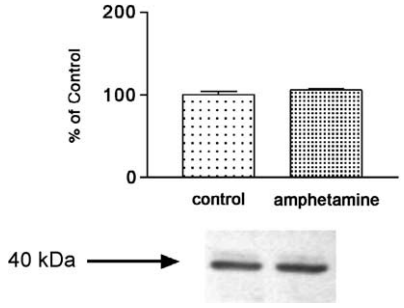

Fig. 4. Effects of chronic D-amphetamine treatment, group C (5 days, 2.5 mg/kg i.p., D-amphetamine, 14-day withdrawal, 0.5 mg/kg i.p., D-amphetamine, 4 h to both treated and control groups) in the rat nucleus accumbens. Data are represented as mean ± S.E.M. ($n=6$). Statistical significance is expressed as $P \leq 0.05$. A representative immunoblot is shown below the bar graph.

3.5. Chronic D-amphetamine administration, group D rats demonstrated an increase of CRP40 levels in the nucleus accumbens only

Group D rats were treated the same way as group C, except decapitation occurred 8 h after the challenge dose of D-amphetamine. There is a significant increase in CRP40 in the nucleus accumbens (40.49 ± 15.91%, $P \leq 0.05$) which is displayed clearly in Fig. 5. The results suggest that the

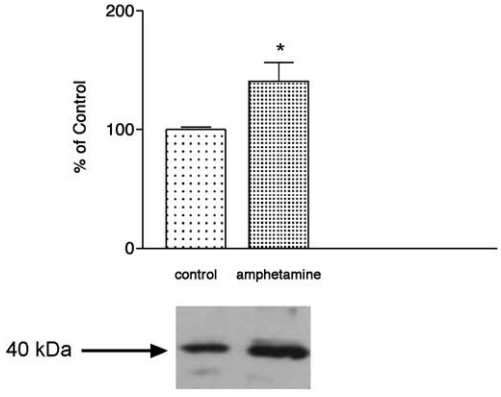

Fig. 5. Effects of chronic D-amphetamine treatment, group D (5 days, 2.5 mg/kg i.p., D-amphetamine, 14-day withdrawal, re-administered 0.5 mg/kg i.p., D-amphetamine, 8 h to both treated and control groups, and decapitated 8 h later. Data are presented as mean ± S.E.M. ($n=6$). Statistical significance is expressed as $*P \leq 0.05$. A representative immunoblot is shown below the bar graph.

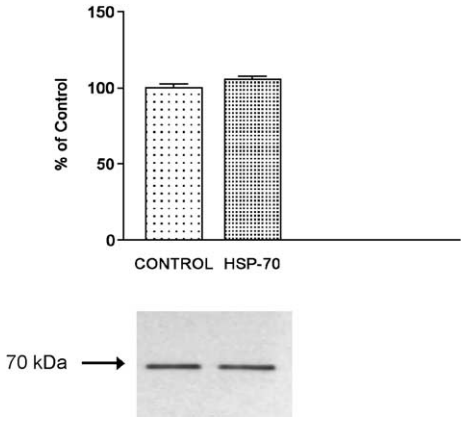

Fig. 6. Effects of chronic D-amphetamine treatment, group A (2.5 mg/kg i.p., D-amphetamine) on Hsp-70 levels in the nucleus accumbens. Data are presented as mean ± S.E.M. ($n = 6$). Statistical significance is expressed as $P \leq 0.05$. A representative immunoblot is shown below the bar graph.

time required for differential expression of CRP40 in the nucleus accumbens was between 4 and 8 h and suggests that neurons in the mesolimbic dopamine system are more readily affected by D-amphetamine treatment, even at extremely low doses.

3.6. Acute and chronic D-amphetamine treatment does not affect expression of constitutive and inducible 70-kDa heat shock proteins

Although CRP40 belongs to a family of heat shock proteins, the effect of D-amphetamine was confined to CRP40 only. There were no changes in either the constitutive or inducible forms of the 70-kDa heat shock protein expression following amphetamine treatment under identical experimental conditions, suggesting that the 70-kDa heat shock protein is not modulated in the same manner as CRP40. The summary of the analysis of the 70-kDa heat shock protein is shown in Figs. 6 and 7.

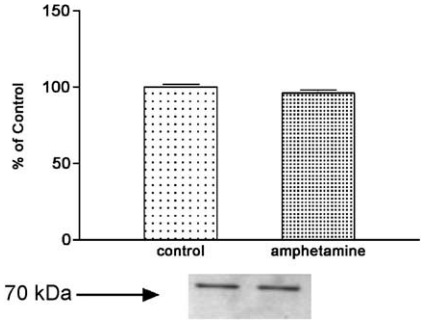

Fig. 7. Effects of chronic D-amphetamine treatment, group A (2.5 mg/kg i.p., D-amphetamine) on Hsc-70 levels in the nucleus accumbens. Data are presented as mean ± S.E.M. ($n = 6$). Statistical significance is expressed as $P \leq 0.05$. A representative immunoblot is shown below the bar graph.

4. Discussion

The purpose of this study was to examine the in vivo differential effects of the indirect dopamine receptor agonist, D-amphetamine, on the expression of CRP40 in various brain regions. The present investigation demonstrated that acute treatment with D-amphetamine (5.0 mg/kg, i.p.) resulted in no change in CRP40 levels in the two brain regions studied: striatum and nucleus accumbens. However, chronic treatment with D-amphetamine resulted in up-regulation of CRP40 levels in the striatum and nucleus accumbens. In addition, possible sensitized challenged rats (0.5 mg/kg, i.p.) demonstrated up-regulation only in the nucleus accumbens. Under identical experimental conditions, both the inducible and constitutive levels of the 70-kDa heat shock protein remained unaltered (Figs. 6 and 7).

Acute dose of D-amphetamine is known to promote high focused stereotypy such as enhanced locomotion and exploratory behaviour (Robinson and Becker, 1986). The predominant action of acute administration of D-amphetamine is to promote the rapid and pronounced release of dopamine from the presynaptic neuron. D-Amphetamine also increases extracellular dopamine levels by inhibiting the dopamine transporter, which functions in the re-uptake of the neurotransmitter into the presynaptic bouton (Kuczenski and Segal, 1992). Increased dopamine levels can activate the dopamine D1 receptor, which elevates cAMP levels via the stimulatory G-protein (Molloy et al., 1986). Sharan et al. (2001) suggest that CRP40 is up-regulated by dopamine D1 receptor-induced increases in cAMP levels (Sharan et al., 2001), which have been shown to regulate gene expression through the activation of various transcription factors (Sheng et al., 1991). However, we found no increase in CRP40 expression in the striatum and nucleus accumbens following acute D-amphetamine treatment, which can increase dopamine levels, as discussed. This phenomenon may be a result of insufficient time allowed for the synthesis and expression of the CRP40 protein and/or the need of repeated drug administration. A similar idea has been suggested by Sharan et al. (2001) to explain their observation of a lack of CRP40 up-regulation following acute haloperidol treatment, which can also significantly increase dopamine levels.

The chronic injections of D-amphetamine (2.5 mg/kg, i.p., 5 days) is known to induce robust behavioural sensitization in rats (Kuczenski et al., 1997). Behavioural sensitization is a progressive increase in the behavioural response to the drug with repeated administration (Kalivas and Stewart, 1991; Robinson and Becker, 1986). Even though no behavioural parameters such as locomotor activity were measured in this study, the phenomenon of behavioural sensitization should be mentioned because the dose regimen used here is known to cause behavioural sensitization with chronic D-amphetamine administration in rats. Neurochemically, researchers have extensively studied the abusive nature of D-amphetamine, because it produces

psychosis similar to paranoid schizophrenia. Past studies have shown that behavioural sensitization and dopamine release may be linked, but neural alterations of the dopamine system and the behavioural sensitization phenomenon are poorly understood (Carboni et al., 2001; Kuczenski et al., 1997).

The effects of chronic D-amphetamine (2.5 mg/kg, i.p., 5 days) treatment on group A rats caused up-regulation of CRP40 in the striatum and nucleus accumbens ($37.46 \pm 8.46\%$ and $27.86 \pm 8.40\%$, respectively, $P \leq 0.05$). It has been hypothesized that lower intermittent doses of D-amphetamine stimulates supersensitized dopamine D1 receptors, activating adenylyl cyclase and the cAMP cascade, involving cAMP response element-binding protein (CREB) and immediate early transcription genes (IEG) (Konradi et al., 1994). The involvement of CREB and chronic pyschostimulant use is believed to be involved in long-term neural and behavioural plasticity (Nestler and Aghajanian, 1997). Because past studies have shown that chronic administration of D-amphetamine induces phosphorylate CREB, we hypothesize that CRP40 expression is regulated by the cAMP cascade, most likely downstream at the immediate early transcription genes (Cole et al., 1995).

In addition, the up-regulation of time-delayed glutamate efflux in the mesolimbic neurons due to chronic D-amphetamine treatment could involve neural alterations and possible neurotoxicity (Eisch and Marshall, 1998; Wolf, 1998). Excitotoxicity due to the efflux of glutamate neurotransmission and the induction of oxidative stress due to excess extracellular dopamine and its quinone metabolites caused by chronic D-amphetamine treatment could result in the induction of the CRP40 molecular chaperones, as a compensatory mechanism against these harmful compounds in the striatum and nucleus accumbens.

Earlier studies have demonstrated that an increase of dopamine release in the striatum and nucleus accumbens occurs in a time-dependent fashion following a D-amphetamine challenge dose after a 14-day drug withdrawal (Vezina, 1993; Paulson and Robinson, 1995). These studies correlate with ours on the assumption that CRP40 is indirectly modulated by dopamine release. Previously sensitized group D rats (2.5 mg/kg, i.p., D-amphetamine, 5 days) that were challenged 14 days later with 0.5 mg/kg, i.p., D-amphetamine demonstrated a CRP40 up-regulation in the nucleus accumbens. The difference between group C and group D was decapitation periods following D-amphetamine (0.5 mg/kg, i.p.) challenged administration (4 and 8 h, respectively). This difference of $40.49 \pm 15.91\%$ ($P \leq 0.05$) in CRP40 up-regulation required at least 4–8 h to be expressed. A possible explanation for CRP40 up-regulation solely in the nucleus accumbens could be due to the supersensitivity of D1 receptors reported in the mesolimbic region originating from the ventral tegmental area in comparison to nigrostrial neurons. In addition, sensitivity of receptors in the nucleus accumbens are further divided into shell and core compartments, which could be responsible for the neural alteration of the dopamine system and could be associated with behavioural sensitization to amphetamine treatment (Cadoni et al., 2000).

Recently, bovine brain CRP40 protein has been cloned and has been demonstrated to have a consensus motif with the 70-kDa heat shock protein (Nair and Mishra, 2001). In addition, a protein sequence bank (GenBank) was utilized, and a comparison analysis using the BLASTP program revealed that CRP40 has a unique protein sequence compared to any known mammalian species. However, levels of constitutive and inducible 70-kDa heat shock proteins were unaffected by D-amphetamine treatment in any of the brain regions studied, in both acute and chronic D-amphetamine-treated groups. This is consistent with Sharan et al. (2001), who demonstrated that both dopamine D1 and D2 antagonists led to no change in levels of constitutive and inducible heat shock proteins. These results suggest that these effects on CRP40 are specific with respect to D-amphetamine treatment.

In conclusion, our results demonstrate that acute treatment with D-amphetamine does not change CRP40 modulation in the striatum and nucleus accumbens. However, chronic D-amphetamine treatment increased CRP40 expression in both the striatum and nucleus accumbens. Possible sensitized and challenged rats showed CRP40 up-regulation in the nucleus accumbens only. Future studies should focus on the mechanism of action of behavioural sensitization and the subcompartments (shell/core) of the nucleus accumbens and its effects on CRP40 differential expression. Finally, D-amphetamine did not have an affect on the expression of the 70-kDa heat shock proteins in any of brain regions studied. Although CRP40 has some homology with the 70-kDa heat shock protein, its specificity to the central nervous system is regulation by dopaminergic agents and lacks cross-reactivity with heat shock protein antibodies; This suggests that CRP40 is quite unique compared to other heat shock proteins. This study provides a framework for future investigations, with respect to the role of CRP40 as a molecular chaperone and its possible links to schizophrenia.

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References

Bear, M.F., Connors, B.W., Paradiso, M.A., 2001. Neuroscience: Exploring The Brain, 2nd ed. Lippincott Williams & Wilkins, Baltimore, MD, p. 969.

Bowyer, J.F., 2000. Neuronal degeneration in the limbic system of weanling rats exposed to saline, hyperthermia or D-amphetamine. Brain Res. 885, 166–171.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cadet, J.L., Brannock, C., 1998. Free radicals and the pathobiology of brain dopamine systems. Neurochem. Int. 32, 117–131.

Cadoni, C., Solinas, M., Di Chiara, G., 2000. Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. Eur. J. Pharmacol. 388, 69–76.

Carboni, E., Spielewoy, C., Vacca, C., Nosten-Bertrand, M., Giros, B., Di Chiara, G., 2001. Cocaine and amphetamine increase extracellular dopamine in the nucleus accumbens of mice lacking the dopamine transporter gene. J. Neurosci. 21, RC141–RC144.

Cole, R.L., Konradi, C., Douglass, J., Hyman, S.E., 1995. Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. Neuron 14, 813–823.

Eisch, A.J., Marshall, J.F., 1998. Methamphetamine neurotoxicity: dissociation of striatal dopamine terminal damage from parietal cortical cell body injury. Synapse 30, 433–445.

Goto, A., Doering, L., Nair, V.D., Mishra, R.K., 2001. Immunohistochemical localization of a 40-kDa catecholamine regulated protein in the nigrostriatal pathway. Brain Res. 900, 314–319.

Halliwell, B., Gutteridge, J.M., Cross, C.E., 1992. Free radicals, antioxidants, and human disease: where are we now? J. Lab. Clin. Med. 119, 598–620.

Heikkila, R.E., Orlansky, H., Mytilineou, C., Cohen, G., 1975. Amphetamine: evaluation of d- and l-isomers as releasing agents and uptake inhibitors for ^{3}H-dopamine and ^{3}H-norepinephrine in slices of rat neostriatum and cerebral cortex. J. Pharmacol. Exp. Ther. 194, 47–56.

Jackson, D.M., Westlind-Danielsson, A., 1994. Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmacol. Ther. 64, 291–370.

Jones, D.C., Gunasekar, P.G., Borowitz, J.L., Isom, G.E., 2000. Dopamine-induced apoptosis is mediated by oxidative stress and Is enhanced by cyanide in differentiated PC12 cells. J. Neurochem. 74, 2296–2304.

Kalivas, P.W., Stewart, J., 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res., Brain Res. Rev. 16, 223–244.

Kandel, E.R., Swartz, J.H., Jessell, T.M., 2000. Principles of Neural Science, 4th ed. McGraw-Hill, New York, NY, p. 1011.

Konradi, C., Cole, R.L., Heckers, S., Hyman, S.E., 1994. Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J. Neurosci. 14, 5623–5634.

Kuczenski, R., 1986. Dose response for amphetamine-induced changes in dopamine levels in push–pull perfusates of rat striatum. J. Neurochem. 46, 1605–1611.

Kuczenski, R., Segal, D.S., 1992. Regional norepinephrine response to amphetamine using dialysis: comparison with caudate dopamine. Synapse 11, 164–169.

Kuczenski, R., Leith, N.J., Applegate, C.D., 1983. Striatal dopamine metabolism in response to apomorphine: the effects of repeated amphetamine pretreatment. Brain Res. 258, 333–337.

Kuczenski, R., Segal, D.S., Todd, P.K., 1997. Behavioural sensitization and extracellular dopamine responses to amphetamine after various treatments. Psychopharmacology (Berl.) 134, 221–229.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lewis, D.A., Lieberman, J.A., 2000. Catching up on schizophrenia: natural history and neurobiology. Neuron 28, 325–334.

Marcotte, E.R., Pearson, D.M., Srivastava, L.K., 2001. Animal models of schizophrenia: a critical review. J. Psychiatry Neurosci. 26, 395–410.

Modi, P.I., Kashyap, A., Nair, V.D., Ross, G.M., Fu, M., Savelli, J.E., Marcotte, E.R., Barlas, C., Mishra, R.K., 1996. Modulation of brain catecholamine absorbing proteins by dopaminergic agents. Eur. J. Pharmacol. 299, 213–220.

Molloy, A.G., O'Boyle, K.M., Pugh, M.T., Waddington, J.L., 1986. Locomotor behaviors in response to new selective D-1 and D-2 dopamine receptor agonists, and the influence of selective antagonists. Pharmacol. Biochem. Behav. 25, 249–253.

Moore, K.E., Von Voigtlander, P.F., 1971. The release of ^{3}H-dopamine from cat brain following electrical stimulation of the substantia nigra and caudate nucleus. Neuropharmacology 10, 733–741.

Nair, V.D., Mishra, R.K., 2001. Molecular cloning, localization and characterization of a 40-kDa catecholamine-regulated protein. J. Neurochem. 76, 1142–1152.

Nestler, E.J., Aghajanian, G.K., 1997. Molecular and cellular basis of addiction. Science 278, 58–63.

Ohtsuka, K., Suzuki, T., 2000. Roles of molecular chaperones in the nervous system. Brain Res. Bull. 53, 141–146.

Paulson, P.E., Robinson, T.E., 1995. Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 19, 56–65.

Robinson, T.E., Becker, J.B., 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. 396, 157–198.

Ross, G.M., McCarry, B.E., Thakur, S., Mishra, R.K., 1993. Identification of novel catecholamine absorbing proteins in the central nervous system. J. Mol. Neurosci. 4, 141–148.

Ross, G.M., McCarry, B.E., Mishra, R.K., 1995. Covalent affinity labeling of brain catecholamine-absorbing proteins using a high-specific-activity substituted tetrahydronaphthalene. J. Neurochem. 65, 2783–2789.

Rutledge, C.O., 1970. The mechanisms by which amphetamine inhibits oxidative deamination of norepinephrine in brain. J. Pharmacol. Exp. Ther. 171, 188–195.

Sharan, N., Nair, V.D., Mishra, R.K., 2001. Modulation of a 40-kDa catecholamine regulated protein by dopamine receptor antagonists. Eur. J. Pharmacol. 413, 73–79.

Sheng, M., Thompson, M.A., Greenberg, M.E., 1991. CREB: a Ca^{2+}-regulated transcription factor phosphorylated by calmodulin-dependent kinases. Science 252, 1427–1429.

Stokes, A.H., Hastings, T.G., Vrana, K.E., 1999. Cytotoxic and genotoxic potential of dopamine. J. Neurosci. Res. 55, 659–665.

Suzuki, T., Usuda, N., Murata, S., Nakazawa, A., Ohtsuka, K., Takagi, H., 1999. Presence of molecular chaperones, heat shock cognate (Hsc) 70 and heat shock proteins (Hsp) 40, in the postsynaptic structures of rat brain. Brain Res. 816, 99–110.

Vallone, D., Picetti, R., Borrelli, E., 2000. Structure and function of dopamine receptors. Neurosci. Biobehav. Rev. 24, 125–132.

Vezina, P., 1993. Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an in vivo microdialysis study in the rat. Brain Res. 605, 332–337.

Von Voigtlander, P.F., Moore, K.E., 1973. Involvement of nigro-striatal neurons in the in vivo release of dopamine by amphetamine, amantadine and tyramine. J. Pharmacol. Exp. Ther. 184, 542–552.

Wolf, M.E., 1998. The role of excitatory amino acids in behavioural sensitization to psychomotor stimulants. Prog. Neurobiol. 54, 679–720.