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Modulation of a 40-kDa catecholamine regulated protein by dopamine receptor antagonists

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Abstract

Previous reports have shown that catecholamine regulated proteins (CRP) are central nervous system specific and covalently bind to catecholamines. In the present study, we report the subcellular localization and differential modulation of a 40-kDa catecholamine regulated protein (CRP40) by dopamine D1 and D2 receptor antagonists. CRP40 was found to be localized with nuclear and synaptosomal/mitochondrial and fractions. Chronic treatment with dopamine D2 receptor antagonist haloperidol in rats significantly increased the levels of CRP40 in the striatum, whereas, chronic *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390) dopamine D1 receptor antagonist administration significantly decreased striatal CRP40 levels. Moreover, acute haloperidol treatment did not alter the levels of CRP40 in any of the brain regions. Despite a sequence homology with the heat shock protein 70 (HSP70), levels of HSP70 remained unchanged after either drug treatment, suggesting a distinct function of CRP40 than HSP70. These results further suggest that CRP40 play an important role in dopaminergic neuronal function and the dopamine D1 receptor-mediated signaling pathway may be involved in the regulation of CRP40. © 2001 Published by Elsevier Science B.V.

Keywords: Catecholamine regulated protein; Dopamine; Heat shock protein; Neurodegenerative disease; Haloperidol; SCH 23390

1. Introduction

Catecholamines reversibly bind to specific receptor proteins present on the surface of target cells. The covalent incorporation of catechols into proteins has also been recognized for some time as a reaction in which many catechols and catecholamines participate (Saner and Thoenen, 1971; Maguire et al., 1974; Scheulen et al., 1975; Rotman et al., 1976; Kato et al., 1986). The oxidation of dopamine ultimately forms reactive oxygen species which lead to cellular oxidative stress and damage a variety of critical biological molecules (Cohen 1983; Cadet and Lohr, 1989; Cadet and Brannock, 1998; Olanow 1993; Hattori et al., 1998; Naoi and Maruyama, 1999; Jones et al., 2000; Stokes et al., 2000).

Oxidative stress secondary to dopamine metabolism has been proposed as a pathogenic factor in the development of neurodegenerative disorders (Cohen, 1983; Ebadi et al., 1996). It is unlikely that dopamine oxidation is the primary pathology of neurodegenerative illnesses, rather, it may be part of a sequence of events that occur during cell death (Jenner, 1998). There has been cumulative evidence showing that aberrations in second messenger responses may be involved in the development of neurological disorders (Treisman et al., 1986; Fici et al., 1997). It has been shown that dopamine D1 receptors mediate the stimulation of adenylyl cyclase whereas dopamine D2 receptors are associated with inhibition of adenylyl cyclase (Spano et al., 1978; Kebabian and Calne, 1979) dopamine D1 and D2 receptor blockade by *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390) and haloperidol, respectively, have been shown to induce differential changes in neuronal second messenger status in the rat striatum (Memo et al., 1987a; Treisman et

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al., 1986; Onali et al., 1985; Rogoz and Dziedzicka-Wasylewska, 1996; Fici et al., 1997).

We have previously reported a unique class of catecholamine regulated proteins (CRPs) which covalently bind to dopamine and structurally related catecholamines (Ross et al., 1993, 1995). Dopamine agonists have been shown to modulate in vivo levels of CRPs in rats (Modi et al., 1996). Recent cloning and characterization of the 40 kDa CRP (CRP40) shows that it has sequence homology with a consensus motif of 70 kDa heat shock protein (HSP70) and may be a mammalian cognate of heat shock proteins. (Nair and Mishra, 1999, 2001).

In order to further understand the role of CRP40 in dopaminergic function, we investigated whether dopamine D1 and D2 receptor antagonists have identical or dissimilar effects on CRP40 since they reciprocally modulate cAMP levels (Kebabian and Calne, 1979; Kebabian et al., 1972; Stoof and Kebabian, 1981) and adenylyl cyclase activity (Onali et al., 1985). In order to specify CRP40's localization in neuronal cells, the levels of these proteins were measured in subcellular fractions. Studies with β-adrenoceptor antagonist propranolol were also carried out to determine whether CRP40 is specific to dopaminergic agents. Furthermore, since CRP40 shares sequence homology with HSP70, parallel studies with HSP70 were performed in order to differentiate these proteins.

2. Materials and methods

2.1. Animals and materials

Male Sprague–Dawley rats (250–300 g) were obtained from Charles River Canada (St. Constant, QC, Canada). Animals were housed in pairs at the McMaster University Central Animal Facility and kept at constant temperature (22°C) and humidity (50%), under a 12-h lights on/off schedule. The rats were handled for 1 week prior to treatment and had free access to food and water throughout the course of experiments. Body weights of the rats were monitored daily and all animal procedures were carried out according to the guidelines approved by the Canadian Council for Animal Care.

Haloperidol was purchased from Sigma (St. Louis, MO, USA). SCH 23390 and propranolol were obtained from Research Biochemicals International (Natik, MA).

2.2. Drug treatment

For dopamine D2 receptor studies, control groups received vehicle and experimental groups received haloperidol (2 mg/kg, i.p.). Group A ($n = 6$) received vehicle. Group B ($n = 6$) received haloperidol daily for 14 days. Group C ($n = 6$) received haloperidol once daily for 2 days. And Group D ($n = 6$) received a single dose of haloperidol. For dopamine D1 receptor studies, Group A ($n = 6$) received vehicle whereas Group B received SCH 23390 (0.5 mg/kg, i.p.) once daily for 14 days. For β-adrenoceptor studies, Group A ($n = 6$) received vehicle and Group B ($n = 6$) received propranolol (5 mg/kg, i.p.) once daily for 14 days. Haloperidol was dissolved in acetic acid and the pH adjusted to 7.4. Further dilution was made in normal saline. SCH 23390 and propranolol were dissolved in normal saline.

At the end of the last drug treatment, each rat was allowed a 24-h washout period except those of Group D receiving haloperidol (decapitated 2 h post-injection). After decapitation, the striatum, frontal cortex and medulla were dissected out and stored at −70°C until further processed for western blotting.

2.3. Sodium dodecylsulfate polyacryl amide gel electrophoresis (SDS-PAGE) and western blotting

Rat striatum, frontal cortex and medulla were weighed and homogenized in Tris buffer (50 mM Tris, 1 mM EDTA, pH 7.4). Protein concentration was determined according to the method of Bradford (1976). Ten micrograms of protein was mixed with sample buffer (0.625 M Tris, 2% SDS, 10% glycerol, 0.05% β-mercaptoethanol, 0.01% bromophenol blue, pH 6.8) and heated in boiling water for 4 min. The proteins were separated by SDS-PAGE (12% acrylamide) (Laemmli, 1970) using running buffer (0.025 M Tris, pH 8.3, 0.2 M glycine, 0.1% SDS) at 100 V for 2 h. Separated proteins were transferred electrophoretically onto nitrocellulose for 1.5 h at 30 V and subsequently blocked in 5% skim milk/TBS-T (0.05 M Tris, 0.15 M NaCl, 0.2% Tween-20, pH 8.5) for 1 h at room temperature. The nitrocellulose membrane was incubated with rabbit anti-CRP40 antibody (1:10,000 dilution) overnight at 4°C then washed three times with TBS-T buffer. Incubation with secondary antibody (HRP-conjugated donkey anti-rabbit antibodies, 1:5000 dilution) proceeded for 1 h at room temperature after which blots were developed by chemiluminescence method and exposed to Kodak X-OMAT film. Levels of CRP40 were quantified using a computerized image analysis system (Northern Exposure, Empix Imaging).

2.4. Isolation of subcellular fractions from the striatum

Rat striata were used to prepare subcellular fractions by differential centrifugation. Rat striatal tissue was homogenized in 10 volumes of chilled buffer A (0.25 M sucrose, 50 mM Tris, 1 mM EDTA, pH 7.4) and centrifuged at $1000 \times g$ for 10 min at 4°C producing supernatant S1. S1 was further centrifuged at $35{,}000 \times g$ for 30 min, producing S2 and precipitate P2. The former step was repeated

for the P2 fraction producing P3 (synaptosome/mitochondria). S2 was further subjected to centrifugation at 50,000 × *g* for 2 h, producing S4 (cytoplasm) and P4 (ribosomes and large macromolecules). The purity of various subcellular fractions were previously established using specific marker enzymes (NADPH-cytochrome C reductase for rough and smooth endoplasmic reticulum, succinate dehydrogenase and glutamate dehydrogenase for mitochondria) (Mishra and Feltham, 1973). CRP40 content was determined in each subcellular fraction by western immunoblotting.

2.5. Isolation of nuclei from the striatum

Nuclei were isolated from rat striatum by sucrose density centrifugation. Tissues were weighed and homogenized in five volumes of chilled buffer B (0.32 M sucrose, 50 mM Tris, 3 mM $CaCl_2$, pH 7.2) followed by centrifugation at 1000 × *g* for 10 min. The pellet was resuspended in five volumes of chilled buffer B and centrifuged at 1000 × *g* for 10 min. The pellet was resuspended in five volumes of chilled buffer C (2.4 M sucrose, 50 mM Tris, 3 mM $CaCl_2$, pH 7.2) and centrifuged at 50,000 × *g* for 1 h. The floating stop plug (containing whole cells, erythrocytes, mitochondria) was removed. The pellet of nuclei was suspended in 500 μl of buffer A. The purity of the nuclear fraction was determined using the phase contrast microscope (Mishra and Feltham, 1973). CRP40 content in the nuclear fraction was determined by western immunoblotting.

2.6. Statistical analysis

Data are presented as mean S.E.M. (Standard Error of the Mean). Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc comparison test.

3. Results

3.1. Dopamine D2 receptor antagonist haloperidol increases CRP40 levels in the striatum but not in frontal cortex and medulla

In order to study the effects of acute and chronic dopamine D2 receptor antagonist treatment on CRP40, animals were treated with haloperidol and the levels of CRP40 in striatum; frontal cortex and medulla were measured by western immunoblotting. The results of acute and chronic treatment with haloperidol in the rat striatum are shown in Fig. 1A and B. Acute treatments (2 h, 2 days) had no effect on CRP40 levels in any of the brain regions examined. However, prolonged exposure to haloperidol (14 days) significantly ($P \leq 0.01$) increased CRP40 in the striatum (26.3 ± 5.0%). There were no effects of these acute and chronic treatments of haloperidol on CRP40 levels in rat frontal cortex and medulla (Fig. 2A) suggesting CRP40's specificity for Dopamine rich regions.

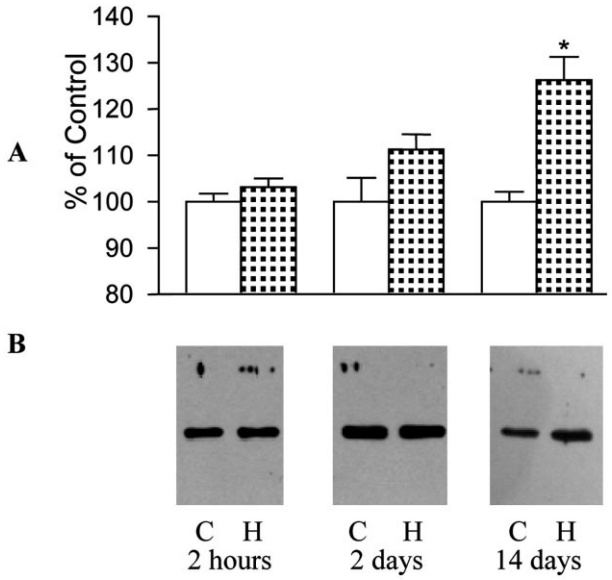

Fig. 1. (A) Effects of acute (2 h, 2 days) and chronic (14 days) haloperidol (H) treatment (2 mg/kg, i.p.) on CRP40 levels in the rat striatum. Bars represent the percentage of control (C) (untreated) values. Data are presented as mean S.E.M. ($n = 6$). A one-way ANOVA was performed followed by Tukey's post hoc comparison test. Statistical significance expressed as: $^{*}P \leq 0.01$. (B) Representative immunoblot shows the effects of the above treatments.

3.2. Dopamine D1 receptor antagonist SCH 23390 decreases CRP40 levels in the striatum but not in frontal cortex and medulla

Results of chronic treatment with D1 receptor antagonist SCH 23390 are shown in Fig. 2B. These results show that SCH 23390 had the opposite effect than haloperidol in the striatum. There was a significant ($P \leq 0.01$) decrease in CRP40 levels (−32.8 ± 9.6%) in this region. However, frontal cortex and medulla showed no change in CRP40 levels after chronic SCH 23390 treatment. The summary of the differential effects of chronic dopamine D1 and D2 receptor antagonists on CRP40 levels in different rat brain regions is shown in Fig. 2C.

3.3. Specificity of CRP40 for the dopaminergic agents

In order to establish the specificity of CRP40 for the dopaminergic system, the effects of the β-adrenoceptor antagonist propranolol on CRP40 levels were examined. Fig. 2D and E show that there was no effect of chronic (14 days) propranolol treatment on CRP40 levels in the striatum suggesting the specificity of dopaminergic agents on this protein.

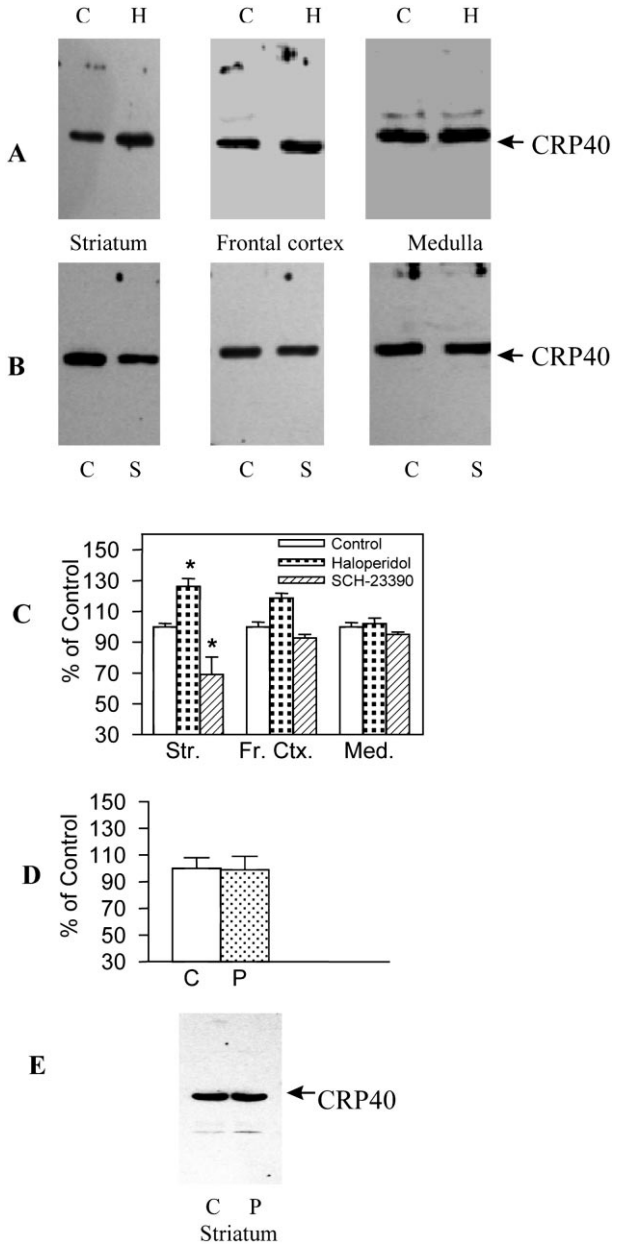

Fig. 2. Representative immunoblot showing the effects of chronic (14 days) (A) haloperidol (H) (2 mg/kg, i.p.) and vehicle (C) and (B) SCH 23390 (S) (0.5 mg/kg, i.p.) treatment on CRP40 levels in different rat brain regions. Summary (C) of the effects of chronic treatment with haloperidol and SCH 23390 on CRP40 levels in different rat brain regions. Bars represent the percentage of control (untreated) values. Data are presented as mean S.E.M. ($n = 6$). A one-way ANOVA was performed followed by Tukey's post hoc comparison test. Statistical significance expressed as: $P \leq 0.01$. Bar graph (D) and corresponding immunoblot (E) displaying effect of chronic (14 days) propranolol (P) treatment (5 mg/kg, i.p.) on CRP40 levels in rat striatum. Str, striatum; Fr. Ctx, frontal cortex; Med, medulla.

3.4. Dopamine D1 receptor and dopamine D2 receptor antagonist do not affect HSP70 levels

Since CRP40 shares a sequence homology with HSP70, (Nair and Mishra 2001) effects of haloperidol and SCH 23390 on HSP70 levels were also examined. The results of chronic haloperidol and SCH 23390 treatment on the constitutive (HSC70) and inducible (HSP70) forms of HSP70 are displayed in Fig. 3A and B. There was no

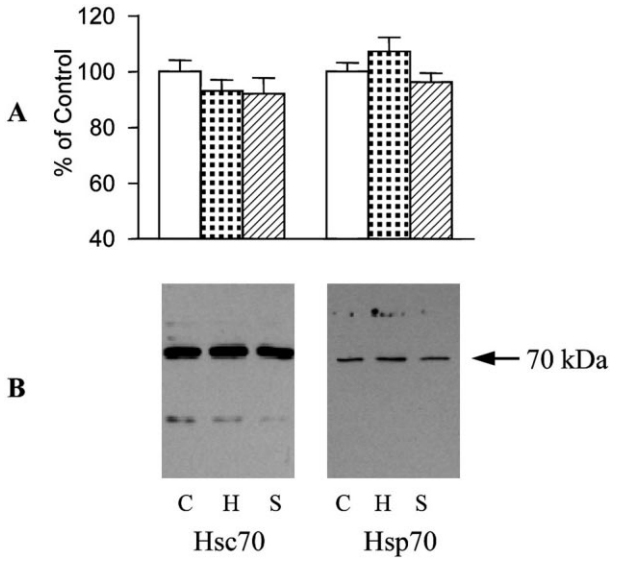

Fig. 3. (A) Effect of chronic treatment with haloperidol (H) (2 mg/kg, i.p) and SCH 23390 (S) (0.5 mg/kg, i.p.) on HSC70 and HSP70 levels in rat striatum. Bars represent the percentage of control (C) (untreated) values. Data are presented as mean S.E.M. ($n = 6$). A one-way ANOVA was performed followed by Tukey's post hoc comparison test. No significance was observed. (B) Representative immunoblot showing the effect of chronic treatment on HSP70 levels in rat striatum.

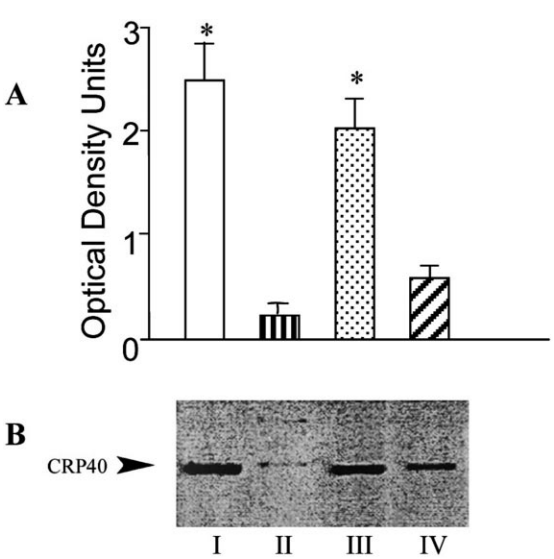

Fig. 4. Bar graph (A) and representative immunoblot (B) showing the subcellular localization of CRP40 in the rat striatum. Data are presented as mean optical density ± S.E.M. ($n = 6$). Lane: I, nuclei; II, cytoplasm; III, synaptosomal/mitochondrial membranes; IV, endoplasmic reticulum/golgi complex/plasma membrane. Statistical analysis was performed using a one-way ANOVA followed by Tukey's post hoc comparison test. Nuclei and synaptosomal/mitochondrial fractions contain higher levels of CRP40 compared to the cytoplasm ($^{*}P \leq 0.001$) and endoplasmic reticulum/golgi complex/plasma membrane fraction ($^{*}P \leq 0.01$).

significant change observed on HSP70 levels suggesting that these proteins are not modulated by dopamine receptor antagonists in the rat brain in a similar fashion as CRP40 under the experimental conditions employed in this study.

3.5. CRP40 is a nuclear and synaptosomal mitochondrial-associated protein

In order to determine the subcellular localization of CRP40, differential centrifugation of striatal rat tissue followed by immunoblotting was performed. Four subcellular regions were isolated (synaptosomes/mitochondria, nuclei, cytoplasm, smooth and rough endoplasmic reticulum) and CRP40 levels were measured by immunodetection in each of the subcellular regions. CRP40 was found to be present in high concentrations in the nuclei and synaptosomes/mitochondria (Fig. 4). Very low levels of CRP40 were detected in the cytoplasm and smooth/rough endoplasmic reticulum.

4. Discussion

We have previously demonstrated that central nervous system specific CRP40 binds to dopamine and other related catecholamines and is modulated by various dopaminergic agents (Ross et al., 1993, 1995; Modi et al., 1996). The objective of the current study was to examine the in vivo effects of dopamine D1 and D2 receptor antagonists, SCH 23390 and haloperidol, respectively, on the levels of CRP40. Studies with propranolol were also performed to establish the specificity of CRP40 for the dopaminergic system. Subcellular fractionation studies were performed in order to further specify CRP40's localization in neuronal cells. Furthermore, since CRP40 contains a consensus motif of HSP70 (Nair and Mishra, 2001), the effects of these drugs on HSP70 levels were also examined in order to differentiate the functional aspect of these proteins.

The present study demonstrated that chronic (14 days) treatment with the selective dopamine D2 receptor antagonist, haloperidol, induced up-regulation of CRP40 in the rat striatum. Conversely, this protein was significantly reduced in the striatum following treatment for 14 days with the dopamine D1 receptor antagonist, SCH 23390. The above effect is specific to the striatum since these drugs failed to alter any changes in CRP40 levels in frontal cortex and medulla. Acute treatments (2 h and 2 days) with the D2 antagonist had no effect on CRP40 levels in any of the brain regions examined. Chronic (14 days) treatment with β-adrenoceptor antagonist propranolol did not alter CRP40 levels in the striatum suggesting that CRP40 is specific for the dopaminergic system. Furthermore, HSP70 levels also remained unchanged upon treatment with dopamine D1 and D2 receptor antagonists in the rat striatum.

Up-regulation and down-regulation of striatal CRP40 may well be related to dopamine D2 and D1 receptor blockade, respectively. There is evidence that a substantial number of striatal neurons co-express both the dopamine D1 and D2 receptor subtypes (Lester et al., 1993). In fact, Surmeier et al. (1996) and Aizman et al. (2000) have shown that functional dopamine D1 and D2 class receptors are co-localized in more than half of all medium spiny neurons present in the striatum. Therefore, dopamine D1–D2 receptor interactions may occur at the level of single postsynaptic neurons (Shetreat et al., 1996; Aizman et al., 2000) and they may function together to mediate the effects of dopamine (Lester et al., 1993). Since dopamine D1 and D2 receptor antagonists had opposite effects on CRP40 levels, it is possible that CRP40 modulation requires functional interaction of both dopamine D1 and D2 dopamine receptors.

Dopamine D2 receptor blockade by haloperidol stimulates dopamine D1 receptors by augmenting the in vivo release of dopamine and increasing the availability of endogenous dopamine at D1 receptors in the rat striatum (Saller and Salama, 1986; Altar et al., 1988). Previous studies have also shown that chronic haloperidol produces enhancement of both D1 receptor mediated stimulation of adenylyl cyclase activity and dopamine mediated inhibition of D2 receptor adenylyl cyclase activity (Memo et al., 1987a). Although the levels of adenylyl cyclase and density of dopamine D1 receptors remain unchanged after chronic haloperidol treatment (Wan et al., 1996), there is a fivefold increase in adenylyl cyclase sensitivity after 14 days haloperidol treatment (Treisman et al., 1986). Since dopamine D2 receptors are linked in an inhibitory manner to adenylyl cyclase (Stoof and Kebabian, 1984), it may be presumed that the enhancement of cAMP activity is due to dopamine D1 receptor alterations (See and Chapman 1994). The stimulation of dopamine D1 receptors and subsequent increase in cAMP levels following chronic neuroleptic treatment may be one of the mechanisms whereby chronic dopamine D2 receptor antagonism with haloperidol up-regulates CRP40 levels in the striatum. Although acute injections of haloperidol have also shown to increase cAMP production in the rat striatum (Kaneko et al., 1992), CRP40 levels remained unchanged perhaps due to the time lag needed for CRP40 expression and synthesis. Nevertheless, findings reported in this manuscript suggest that there may be a positive correlation between CRP40 levels and adenylol cyclase activity in dopaminergic neurons in the striatum. This increase in dopamine D1 receptor activity, cAMP levels and CRP40 might suggest the possible involvement of the dopamine D1 receptor signalling pathway in CRP40 regulation. There is, however, a possibility that CRP40 may recruit its own signalling pathway, which may or may not be coupled with dopamine receptors.

Failure of dopamine D1 and D2 receptor antagonists to modulate CRP40 levels in the rat frontal cortex and medulla may be due to an absence of functional modification of

dopamine receptors in these areas after chronic treatment with antagonists. It has been shown that chronic blockade of either dopamine D1 or dopamine D2 receptors does not affect adenylyl activity and cAMP levels in rat frontal cortex (Memo et al., 1987b). In addition, chronic treatment with neuroleptic drugs results in tolerance to the ability of neuroleptics to increase dopamine turnover in the striatum but not in frontal cortex (Lerner et al., 1977; Scatton et al., 1976). This lack of tolerance may be related to the lack of changes in receptor function. Therefore, the absence of functional receptor modification and tolerance in the frontal cortex and medulla may be related with the lack of changes in CRP40 levels following haloperidol in these regions. Failure for chronic propranolol treatment to alter CRP40 levels in the striatum further establishes the specificity of CRP40 for the dopaminergic system.

Although the CRP40 cDNA contains a domain that has sequence homology with a consensus motif of HSP70 (Nair and Mishra, 2001), the levels of constitutive and inducible HSPs were unaffected by both dopamine D1 and D2 receptor antagonists. Nair and Mishra (1999, 2000) have shown that elevated temperatures increased HSP70 expression, however, incubation of primary neuronal cultures with haloperidol had no affect on the levels of these proteins. Other studies have shown a correlation between cAMP levels and HSPs in a variety of cell types. For example, Pizurki and Polla (1994) demonstrated that cAMP has a direct enhancing effect on the expression of HSPs, however in myeloid cells, cAMP increasing agents decreased the basal level of HSP70 (Vilaboa et al., 1995). In line with these observations, it is possible that dopaminergic-regulated cAMP levels may be involved in CRP40 up-regulation.

In conclusion, the results reported here suggest that CRP40 is specifically increased in response to blockade of dopamine D2 receptors by haloperidol in the striatum of rat brain. Furthermore, the dopamine D1 receptor linked signalling pathway may be involved in CRP40 regulation. Although CRP40 has some homology with HSP70, its regulation by dopaminergic agents, membrane localization, central nervous system specificity and lack of cross-reactivity with HSP antibodies suggest that CRP40 may have a different function than that ascribed to HSPs and may play an important role in dopaminergic function in the central nervous system.

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References

Aizman, O., Brismar, H., Uhlen, P., Zettergen, E., Levey, A.I., Frossberg, H., Greengard, P., Aperia, A., 2000. Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. Nat. Neurosci. 3, 226–230.

Altar, C.A., Boyar, B., Wasley, A., Gerhardt, S.C., Liebman, J.M., Wood, P.L., 1988. Dopamine neurochemical profile of atypical antipsychotics resembles that of D1 antagonist. Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 162–168.

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 pathology of brain dopamine systems. Neurochem. Int. 32, 117–131.

Cadet, J.L., Lohr, J.B., 1989. Possible involvement of free radicals in neuroleptic induced movement disorders. Ann. N. Y. Acad. Sci. 570, 176–185.

Cohen, G., 1983. Pathophysiology of Parkinson's disease: biochemical aspects of dopamine neuron senescence. J. Neural Transm. 19, 89–103.

Ebadi, M., Srinivasan, S.K., Baxi, M.D., 1996. Oxidative stress and antioxidant therapy in Parkinson's disease. Prog. Neurobiol. 48, 1–19.

Fici, G.J., Wu, H., Von Voigtlander, P.F., Sethy, V.H., 1997. D1 dopamine receptor activity of anti-Parkinsonian drugs. Life Sci. 60, 1597–1603.

Hattori, A., Luo, Y., Umegaki, H., Munoz, J., Roth, G.S., 1998. Intrastriatal injections of dopamine result in DNA damage and apoptosis in rats. NeuroReport 9, 2569–2572.

Jenner, P., 1998. Oxidative mechanisms in nigral cell death in Parkinson's disease. Mov. Disord. 13, 24–34.

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.

Kaneko, M., Sato, K., Horikoshi, R., Yaginuma, M., Yaginuma, N., Shiragata, M., Kumashiro, H., 1992. Effect of haloperidol on cyclic AMP and inositol triphosphate in rat striatum in vivo. Prostaglandins Leukotrienes, Essent. Fatty Acids 46, 53–57.

Kato, T., Ito, S., Fujita, K., 1986. Tyrosine-catalyzed binding of 3,4-dihydroxyphenylalanine with proteins through the sulfhydryl group. Biochem. Biophys. Acta. 881, 415–421.

Kebabian, J.W., Calne, D.B., 1979. Multiple receptors for dopamine. Nature 277, 93–96.

Kebabian, J.W., Petzold, G.L., Greengard, P., 1972. Dopamine sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor". Proc. Nat. Acad. Sci. U. S. A. 69, 2145–2149.

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

Lerner, P., Nose, P.N., Gordon, E.K., Lovenberg, W., 1977. Haloperidol: effect of long-term treatment on rat striatal dopamine synthesis and turnover. Science 197, 181–183.

Lester, J., Fink, S., Aronin, N., DiFiglia, M., 1993. Colocalization of D1 and D2 dopamine receptor mRNAs in striatal neurons. Brain Res. 621, 106–110.

Maguire, M.E., Goldman, P.H., Gilman, A.G., 1974. The reaction of [^{3}H]norepinephrine with particulate fraction of cells responsive to catecholamines. Mol. Pharmacol. 10, 563–581.

Memo, M., Pizzi, M., Missale, C., Carruba, M.O., Spano, P., 1987a. Modification of the function of D1 and D2 dopamine receptors in striatum and nucleus accumbens of rats chronically treated with haloperidol. Neuropharmacology 26, 477–480.

Memo, M., Pizzi, M., Nisoli, E., Missale, C., Carruba, M.O., Spano, P., 1987b. Repeated administration of (−)sulpride and SCH 23390 differentially up-regulate D1 and D2 dopamine receptor function in rat

mesostriatal areas but not in cortical-limbic brain regions. Eur. J. Pharmacol. 138, 45–51.

Mishra, R.K., Feltham, L.A.W., 1973. Effect of adrenalectomy and adrenocortical hormones on RNA turnover in a variety of subcellular fractions of rat liver. Can. J. Biochem. 51, 920–930.

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.

Nair, V.D., Mishra, R.K., 1999. Cloning and characterization of a brain specific 40 kDa catecholamine regulated protein. Soc. Neurosci. 794–797.

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

Naoi, M., Maruyama, W., 1999. Cell death of dopamine neurons in aging and Parkinson's disease. Mech. Ageing Dev. 111, 175–188.

Olanow, C.W., 1993. A free radical hypothesis for neurodegeneration. T.I.N.S. 16, 439–444.

Onali, P., Olianas, M.C., Gessa, G.L., 1985. Characterization of dopamine receptors mediating inhibition of adenylate cyclase activity in rat striatum. Mol. Pharmacol. 28, 138–145.

Pizurki, L., Polla, B.S., 1994. cAMP modulates stress protein synthesis in human monocytes-macrophages. J. Cell. Physiol. 161, 169–177.

Rogoz, R., Dziedzicka-Wasylewska, M., 1996. Comparison of the effects of antidepressant drugs on the level of cAMP in the rat striatum and nucleus accumbens septi. Pol. J. Pharmacol. 48, 481–487.

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.

Rotman, A., Daly, J.W., Creveling, C.R., 1976. Oxygen-dependent reactions of 6-hydroxydopamine,5,6-hydroxytryptamine and related compounds with proteins in vitro: a model for cytotoxicity. Mol. Pharmacol. 12, 887–899.

Saller, C., Salama, A.I., 1986. D1 and D2 dopamine receptor blockade: interactive effects in vitro and in vivo. J. Pharmacol. Exp. Ther. 236, 714–720.

Saner, A., Thoenen, H., 1971. Model experiments on the molecular mechanism of action of 6-hydroxydopamine. Mol. Pharmacol. 7, 147–154.

Scatton, B., Glowinski, J., Julou, L., 1976. Dopamine metabolism in the mesolimbic and mesocortical dopaminergic systems after single or repeated administrations of neuroleptics. Brain Res. 109, 184–189.

Scheulen, M., Wollenberg, P., Bolt, H.M., Kappus, H., Remmer, H., 1975. Irreversible binding of DOPA and dopamine metabolites to protein by rat liver microsomes. Biochem. Biophys. Res. Commun. 66, 1369–1400.

See, R.E., Chapman, M.A., 1994. The consequences of long-term antipsychotic drug administration of basal ganglia neuronal function in laboratory animals. Crit Rev. Neurobiol. 8, 85–124.

Shetreat, M.E., Lin, L., Wong, A.C., Rayport, S., 1996. Visualization of D1 dopamine receptors on living nucleus accumbens neurons and their colocalization with D2 receptors. J. Neurochem. 66, 1475–1482.

Spano, P.F., Govoni, S., Trabucchi, M., 1978. Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. Adv. Biochem. Psychopharmacol. 19, 155–165.

Stokes, A.H., Lewis, D.Y., Lash, L.H., Jerome III, W.G., Grant, K.W., Aschner, M., Vrana, K.E., 2000. Dopamine toxicity in neuroblastoma cells: role of glutathione depletion by L-BSO and apoptosis. Brain Res. 858, 1–8.

Stoof, J.C., Kebabian, J.W., 1981. Opposing roles for D1 and D2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature 294, 366–368.

Stoof, J.C., Kebabian, J.W., 1984. Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci. 35, 2281–2296.

Surmeier, D.J., Song, W.J., Yan, Z., 1996. Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. J. Neurosci. 16, 6579–6591.

Treisman, G.J., Muirhead, N., Gnegy, M.E., 1986. Increased sensitivity of adenylate cyclase activity in the striatum of the rat to calmodulin and GppNHp after chronic treatment with haloperidol. Neuropharmacology 25, 587–595.

Vilaboa, N.E., Calle, C., Perez, C., De Blas, E., Garcia-Bermejo, L., Aller, P., 1995. cAMP increasing agents prevent the stimulation of heat-shock protein 70 (HSP70) gene expression by cadmium chloride in human myeloid cell lines. J. Cell Sci. 108, 2877–2883.

Wan, D.C., Dean, B., Pavey, G., Copolov, D.L., 1996. Treatment with haloperidol or clozapine causes changes in dopamine receptors but not adenylate cyclase or protein kinase C in the rat forebrain. Life Sci. 59, 2001–2008.