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Effect of Δ FosB Overexpression on Opioid and Cannabinoid Receptor-Mediated Signaling in the Nucleus Accumbens

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Abstract

The stable transcription factor Δ FosB is induced in the nucleus accumbens (NAc) by chronic exposure to several drugs of abuse, and transgenic expression of Δ FosB in the striatum enhances the rewarding properties of morphine and cocaine. However, the mechanistic basis for these observations is incompletely understood. We used a bitransgenic mouse model with inducible expression of Δ FosB in dopamine D₁ receptor/dynorphin-containing striatal neurons to determine the effect of Δ FosB expression on opioid and cannabinoid receptor signaling in the NAc. Results showed that mu opioid-mediated G-protein activity and inhibition of adenylyl cyclase were enhanced in the NAc of mice that expressed Δ FosB. Similarly, kappa opioid inhibition of adenylyl cyclase was enhanced in the Δ FosB expressing mice. In contrast, cannabinoid receptor-mediated signaling did not differ between mice overexpressing Δ FosB and control mice. These findings suggest that opioid and cannabinoid receptor signaling are differentially modulated by expression of Δ FosB, and indicate that Δ FosB expression might produce some of its effects via enhanced mu and kappa opioid receptor signaling in the NAc.

Keywords

G-protein; adenylyl cyclase; striatum

1. Introduction

Opioid receptors and cannabinoid CB₁ receptors (CB₁R) are the neurobiological targets for two widely used drug classes that include morphine, heroin and prescription opioids, and marijuana (Δ^9 -tetrahydrocannabinol (THC)), respectively. The acute effects of opioids and cannabinoids are mediated by G-protein-coupled receptors that activate primarily G_{i/o} proteins and produce downstream effector responses such as inhibition of adenylyl cyclase

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(Childers, 1991, Childers, et al., 1992, Howlett, et al., 2002). The motor, memory impairing and psychoactive effects of Δ^9 -THC are produced by CB₁R (Huestis, et al., 2001, Zimmer, et al., 1999), which are widely distributed in the brain, with high levels in the basal ganglia, hippocampus and cerebellum (Herkenham, et al., 1991). The analgesic and rewarding effects of most clinically relevant and abused opioid drugs are mediated mainly by mu opioid receptors (MOR) (Matthes, et al., 1996), which are enriched in the limbic system and brainstem (Mansour, et al., 1994). The mesolimbic system, composed of dopaminergic projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc), plays an important role in the rewarding effects of opioids and cannabinoids (Bozarth and Wise, 1984, Vaccarino, et al., 1985, Zangen, et al., 2006), as well as other drugs of abuse (Koob and Volkow, 2010). Moreover, endogenous opioid and cannabinoid systems are involved in the rewarding effects of multiple classes of psychoactive drugs (Maldonado, et al., 2006, Trigo, et al., 2010). Thus, it is important to elucidate mechanisms by which opioid and CB₁R signaling are regulated in the NAc.

A central question in the drug abuse field has been to identify proteins that mediate the transition from acute to long-term effects of psychoactive drugs. The AP-1 transcription factor Δ FosB is particularly interesting because it is a stable truncated splice variant product of the *fosb* gene that accumulates upon repeated exposure to drugs of abuse or natural rewards (McClung, et al., 2004, Nestler, 2008, Nestler, et al., 1999). We have found that Δ FosB is induced in the brain following repeated exposure to morphine, Δ^9 -THC, cocaine or ethanol, with each drug producing a unique regional pattern of Δ FosB expression (Perrotti, et al., 2008). A consistent finding across drugs was that Δ FosB was highly induced in the striatum, where all four drugs induced Δ FosB in the NAc core and all except Δ^9 -THC significantly induced expression in the NAc shell and caudate-putamen. Pharmacological studies showed that co-administration of the dopamine D₁ receptor (D₁R) antagonist SCH 23390 blocked Δ FosB induction in the NAc and caudate-putamen following intermittent cocaine or morphine administration, suggesting the potential importance of D₁R-expressing neurons (Muller and Unterwald, 2005, Nye, et al., 1995). The effect of Δ FosB induction on drug-mediated behaviors has been investigated using bitransgenic mice that express Δ FosB in specific neuronal populations of the NAc and dorsal striatum (Chen, et al., 1998). Mice that express Δ FosB in dynorphin/D₁R positive neurons in the NAc and dorsal striatum (line 11A) show altered responses to drugs of abuse, notably enhanced sensitivity to the rewarding effects of cocaine or morphine (Colby, et al., 2003, Kelz, et al., 1999, Zachariou, et al., 2006). These alterations occurred in the absence of changes in the levels of MOR or various G-protein subunits. However, dynorphin mRNA levels were reduced in the NAc of Δ FosB expressing mice (Zachariou, et al., 2006), suggesting that one target of Δ FosB is a gene encoding an endogenous opioid peptide. Δ FosB induction might also produce behavioral changes by regulating receptor signaling in the NAc, but this possibility has not been investigated. Therefore, the present studies used the bitransgenic mouse model to determine whether overexpression of Δ FosB in dynorphin/D₁R containing striatal neurons alters MOR-mediated G-protein activity and MOR- and KOR-mediated adenylyl cyclase inhibition in the NAc. The effect of Δ FosB on CB₁R-mediated G-protein activity was also assessed because Δ^9 -THC administration induces Δ FosB in the NAc (Perrotti, et al., 2008) and the endocannabinoid system is known to regulate brain reward circuits (Gardner, 2005, Maldonado, et al., 2006), but the effect of Δ FosB on the endocannabinoid system has not been investigated.

2. Materials and Methods

2.1. Reagents

[³⁵S]GTP γ S (1250 Ci/mmol), [α -³²P]ATP (800 Ci/mmol) and [³H]cAMP (26.4 Ci/mmol) were purchased from PerkinElmer (Shelton, CT). ATP, GTP, GDP, cAMP, bovine serum

albumin, creatine phosphokinase, papaverine, imidazole and WIN-55212-2, were purchased from Sigma Aldrich (St. Louis, MO). GTP γ S was purchased from Roche Diagnostic Corporation (Chicago, IL). DAMGO was provided by the Drug Supply Program of the National Institute on Drug Abuse (Rockville, MD). Econo-1 scintillation fluid was obtained from Fisher Scientific (Norcross, GA). Ecolite scintillation fluid was obtained from ICN (Costa Mesa, CA). All other chemicals were obtained from Sigma Aldrich or Fisher Scientific.

2.2. Mice

Male bitransgenic mice derived from NSE-tTA (line A) $\times$ TetOp- Δ FosB (line 11) were generated as described in Kelz et al. (Kelz, et al., 1999). Bitransgenic mice were conceived and raised on doxycycline (100 μ g in drinking water) to suppress transgene expression. At 8 weeks of age, doxycycline was omitted from the water for half of the mice to allow transgene expression, whereas remaining mice were maintained on doxycycline to suppress the transgene. Brains were collected 8 weeks later, the time at which transcriptional effects of Δ FosB are maximal (McClung and Nestler, 2003). A second transgenic mouse line was used in which Δ c-Jun, a dominant negative antagonist of c-Jun, is expressed in D₁R/dynorphin and D₂R/enkephalin cells of the striatum, hippocampus and parietal cortex (Peakman, et al., 2003). C-Jun and related Jun family proteins dimerize with Fos family proteins and bind to the AP-1 site of target genes to regulate transcription. However, truncation of the N-terminus of c-Jun (Δ c-Jun) renders the complex transcriptionally inactive and able to obstruct the DNA binding of active AP-1 complexes. Male bitransgenic mice derived from NSE-tTA (line A) $\times$ TetOp-FLAG- Δ c-Jun (line E) were generated as described in Peakman et al. (Peakman, et al., 2003). Bitransgenic mice were conceived and raised on doxycycline (100 μ g in drinking water) to suppress transgene expression. Pups were weaned at 3 weeks, genotyped, and separated into groups, with half maintained on doxycycline-containing water and half on regular drinking water to induce FLAG- Δ c-Jun expression. Brains were collected 6 weeks later, the time at which maximal levels of FLAG- Δ c-Jun have been measured (Peakman, et al., 2003). All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Membrane Preparation

Brains were stored at -80°C until the day of the assay. Prior to assay, each brain was thawed, and the NAc was dissected on ice. Each sample was homogenized in 50 mM Tris-HCl, 3 mM MgCl₂, 1 mM EGTA, pH 7.4 (membrane buffer) with 20 strokes from a glass homogenizer at 4°C . The homogenate was centrifuged at $48,000 \times g$ at 4°C for 10 min, resuspended in membrane buffer, centrifuged again at $48,000 \times g$ at 4°C for 10 min and resuspended in 50 mM Tris-HCl, 3 mM MgCl₂, 0.2 mM EGTA, 100 mM NaCl, pH 7.4 (assay buffer). Protein levels were determined by the method of Bradford (Bradford, 1976) using bovine serum albumin (BSA) as the standard.

2.4. Agonist-Stimulated [^{35}S]GTP γ S Binding

Membranes were pre-incubated for 10 minutes at 30°C with adenosine deaminase (3 mU/ml) in assay buffer. Membranes (5–10 μ g protein) were then incubated for 2 hr at 30°C in assay buffer containing 0.1% (w/v) BSA, 0.1 nM [^{35}S]GTP γ S, 30 μ M GDP and adenosine deaminase (3 mU/ml) with and without appropriate concentrations of DAMGO or WIN55,212-2. Nonspecific binding was measured with 20 μ M GTP γ S. The incubation was terminated by filtration through GF/B glass fiber filters, followed by 3 washes with 3 ml ice-cold 50 mM Tris-HCl, pH 7.4. Bound radioactivity was determined by liquid scintillation spectrophotometry after overnight extraction of the filters in Econo-1 scintillation fluid.

2.5. Adenylyl Cyclase Assay

Membranes (5–25 μ g protein) were preincubated with adenosine deaminase as described above, then incubated for 15 min at 30°C in the presence or absence of 1 μ M forskolin, with or without DAMGO, U50,488H or WIN55,212-2, in assay buffer containing 50 μ M ATP, [α - 32 P]ATP (1.5 μ Ci), 0.2 mM DTT, 0.1% (w/v) BSA, 50 μ M cyclic AMP, 50 μ M GTP, 0.2 mM papaverine, 5 mM phosphocreatine, 20 units/ml creatine phosphokinase and adenosine deaminase (3 mU/ml) in a final volume of 100 μ l. Under these conditions, total [α - 32 P]cAMP recovered was generally less than 1% of the total amount of added [α - 32 P]ATP in each sample. The reaction was terminated by boiling for 3 min and [32 P]Cyclic AMP was isolated by the dual column (Dowex and alumina) method of Salomon (Salomon, 1979). [3 H] cAMP (10,000 dpm) was added to each tube prior to column chromatography as an internal standard. Radioactivity was determined by liquid scintillation spectrophotometry (45% efficiency for 3 H) after 4.5 ml of the eluate were dissolved in 14.5 ml of Ecolite scintillation fluid.

2.6. Data analysis

Unless otherwise indicated, data are reported as mean values $\pm$ S.E. of 4–8 separate experiments, each of which was performed in triplicate. Net-stimulated [35 S]GTP γ S binding is calculated as agonist-stimulated binding minus basal binding. Net forskolin-stimulated adenylyl cyclase activity is defined as forskolin-stimulated activity – basal activity (pmol/mg/min). Percent inhibition of forskolin-stimulated adenylyl cyclase activity is defined as (net forskolin-stimulated activity in the absence of agonist – net forskolin-stimulated activity in the presence of agonist/net forskolin-stimulated activity in the absence of agonist) $\times$ 100. All curve-fitting and statistical analyses were performed using Prism 4.0c (GraphPad Software, Inc., San Diego, CA). Concentration-effect curves were analyzed by iterative non-linear regression to obtain EC₅₀ and E_{max} values. Statistical significance of the concentration-effect data was determined by two-way analysis of variance (ANOVA), using agonist dose and gene induction (on or off) as the main factors. Statistical significance of curve-fit values (E_{max} or EC₅₀) was determined by the non-paired two-tailed Student's t-test, using Welch's correction or square root transformation of the data where necessary to correct for unequal variances (detected by F-test) in EC₅₀ values.

3. Results

3.1. Effect of Δ FosB expression on opioid and cannabinoid receptor-mediated G-protein activation

To determine whether MOR- or CB₁R-mediated G-protein activation was altered by inducible transgenic expression of Δ FosB in the NAc, agonist-stimulated [35 S]GTP γ S binding was examined in isolated membranes prepared from this region of bitransgenic mice conditionally expressing (Δ FosB on) or not expressing (Δ FosB off) the Δ FosB transgene. The MOR-selective enkephalin analog DAMGO was used to activate MOR and the cannabinoid aminoalkylindole WIN55,212-2 was used to activate CB₁R. These ligands were previously shown to be full agonists at MOR and CB₁R, respectively (Breivogel, et al., 1998, Selley, et al., 1997). It was not feasible to examine KOR-mediated G-protein activity because the signal is too low in rodent brain (Childers, et al., 1998). Results showed concentration-dependent stimulation of G-protein activity by both DAMGO and WIN55,122-2 in NAc from Δ FosB off and Δ FosB on mice (Figure 1). For DAMGO-stimulated activity (Figure 1A), two-way ANOVA of the concentration-effect data revealed significant main effects of Δ FosB status ($p < 0.0001$, $F = 22.12$, $df = 1$) and DAMGO concentration ($p < 0.0001$, $F = 29.65$, $df = 5$) with no significant interaction ($p = 0.857$, $F = 0.387$, $df = 5$). Nonlinear regression analysis of the concentration-effect curves revealed a significantly greater DAMGO E_{max} value in Δ FosB on mice ($E_{\text{max}} = 73 \pm 5.2\%$ stimulation)

relative to Δ FosB off mice ($E_{\max} = 56 \pm 4.1\%$ stimulation; $p < 0.05$ different from Δ FosB on mice by Student's t-test). DAMGO EC_{50} values were not different between Δ FosB on and Δ FosB off mice (302 ± 72 nM versus 212 ± 56 nM, respectively, $p = 0.346$).

In contrast to results obtained with the MOR agonist DAMGO, no Δ FosB status-dependent differences in G-protein activation were observed with the cannabinoid agonist WIN55,212-2 (Figure 1B). Two-way ANOVA of the WIN55,212-2 concentration-effect data revealed a significant main effect of WIN55,212-2 concentration ($p < 0.0001$, $F = 112.4$, $df = 7$), but not of Δ FosB status ($p = 0.172$, $F = 1.90$, $df = 1$) and there was no interaction ($p = 0.930$, $F = 0.346$, $df = 7$). Similarly, there was no effect of Δ FosB status on WIN55,212-2 $E_{\max}$ values ($103 \pm 6\%$ versus $108 \pm 8\%$ stimulation in Δ FosB on and off mice, respectively, $p = 0.813$ by Student's t-test) or EC_{50} values (103 ± 20 nM versus 170 ± 23 nM in Δ FosB on and off mice, respectively, $p = 0.123$).

Based on the shape of the curves and the fact that our previous studies have shown biphasic WIN55,212-2 concentration-effect curves in brain (Breivogel, et al., 1999, Breivogel, et al., 1998), the WIN55,212-2 curves were also analyzed using a two-site model. Analysis of the averaged data showed a slight improvement in goodness of fit using the two-site model ($R^2 = 0.933$ and 0.914 , sum of squares = 3644 and 5463 in Δ FosB on and off mice, respectively) compared to the single-site model ($R^2 = 0.891$ and 0.879 , sum of squares = 6561 and 6628 in Δ FosB on and off mice, respectively). However, no significant differences were found between Δ FosB on and off mice in either the $E_{\max}$ or EC_{50} values of the high or low potency sites (Supplementary Table 1), although there was a trend towards a lower EC_{50} value at the high potency site in mice with Δ FosB on ($EC_{50}^{high} = 28.0 \pm 10.6$ nM) compared to those with Δ FosB off ($EC_{50}^{high} = 71.5 \pm 20.2$ nM; $p = 0.094$). Moreover, there was no effect of Δ FosB status on basal [35 S]GTP γ S binding in NAc membranes (253 ± 14 versus 226 ± 14 fmol/mg in Δ FosB on and off mice, respectively, $p = 0.188$). These data indicate that inducible transgenic expression of Δ FosB in the NAc of mice increased MOR-mediated G-protein activation without significantly affecting CB $_1$ R-mediated or basal G-protein activity.

3.2. Effect of Δ FosB on opioid and cannabinoid receptor-mediated inhibition of adenylyl cyclase

To evaluate the effect of inducible transgenic expression of Δ FosB on modulation of downstream effector activity by MOR and CB $_1$ R, inhibition of 1 μ M forskolin-stimulated adenylyl cyclase activity was examined in NAc membranes. In addition to MOR- and CB $_1$ R-mediated inhibition of adenylyl cyclase activity, effects of KOR activity were also examined using the KOR-selective full agonist U50,488 (Zhu, et al., 1997), because previous results showed that dynorphin mRNA was a target of Δ FosB in the bitransgenic model (Zachariou, et al., 2006). Results showed that DAMGO, U50,488 and WIN55,212-2 each produced concentration-dependent inhibition of adenylyl cyclase activity in both Δ FosB off and Δ FosB on mice (Figure 2). Two-way ANOVA of DAMGO concentration-effect data (Figure 2A) revealed significant main effects of Δ FosB status ($p = 0.0012$, $F = 11.34$, $df = 1$) and DAMGO concentration ($p < 0.0001$, $F = 29.61$, $df = 6$), but no significant interaction ($p = 0.441$, $F = 0.986$, $df = 6$). Nonlinear regression analysis of DAMGO concentration-effect curves revealed a significantly lower DAMGO EC_{50} value in Δ FosB on mice (101 ± 11 nM) compared to Δ FosB off mice (510 ± 182 nM, $p < 0.05$ by student's t-test). However, there was no significant difference in DAMGO $E_{\max}$ values ($20.9 \pm 1.26\%$ versus $19.8 \pm 1.27\%$ inhibition in Δ FosB on and off mice, respectively, $p = 0.534$).

KOR-mediated adenylyl cyclase inhibition also differed as a function of inducible transgenic expression of Δ FosB (Figure 2B). Two-way ANOVA of U50,488 concentration-effect data showed significant main effects of Δ FosB status ($p = 0.0006$, $F = 14.53$, $df = 1$) and

U50,488 concentration ($p < 0.0001$, $F = 26.48$, $df = 3$), with no significant interaction ($p = 0.833$, $F = 0.289$, $df = 3$). Nonlinear regression analysis of concentration-effect curves revealed a greater U50,488 $E_{\max}$ value in Δ FosB on mice ($18.3 \pm 1.14\%$ inhibition) compared to Δ FosB off mice ($12.5 \pm 2.03\%$ inhibition; $p < 0.05$ different from Δ FosB on by Student's *t*-test), with no significant difference in U50,488 EC_{50} values (310 ± 172 nM versus 225 ± 48 nM in Δ FosB on and off mice, respectively, $p = 0.324$).

In contrast to effects observed with MOR and KOR, there was no significant effect of inducible transgenic Δ FosB expression on inhibition of adenylyl cyclase by the cannabinoid agonist WIN55212-2 (Figure 2C). Two-way ANOVA of WIN55,212-2 concentration-effect data showed a significant effect of drug concentration ($p < 0.0001$, $F = 23.6$, $df = 2$), but not of Δ FosB status ($p = 0.735$, $F = 0.118$, $df = 1$) nor was there a significant interaction ($p = 0.714$, $F = 0.343$, $df = 2$). Furthermore, there was no effect of Δ FosB status on basal or forskolin-stimulated adenylyl cyclase activity in the absence of any agonist. Basal adenylyl cyclase activity was 491 ± 35 pmol/mg/min in Δ FosB on mice compared to 546 ± 44 in Δ FosB off mice ($p = 0.346$ by Student's *t*-test). Likewise, adenylyl cyclase activity in the presence of 1μ M forskolin was 2244 ± 163 pmol/mg/min in Δ FosB on mice versus 2372 ± 138 pmol/mg/min in Δ FosB off mice ($p = 0.555$).

3.3. Effect of Δ cJun on opioid and cannabinoid receptor-mediated inhibition of adenylyl cyclase

Because inducible transgenic expression of Δ FosB enhanced inhibitory signal transduction from MOR and KOR to adenylyl cyclase in the NAc, it was of interest to determine whether a dominant negative inhibitor of Δ FosB-mediated transcription would modulate opioid receptor signaling in an opposite manner. To address this question, inhibition of forskolin-stimulated adenylyl cyclase activity by DAMGO and U50,488 was examined in membranes prepared from the NAc of bitransgenic mice conditionally expressing Δ cJun. The results showed no significant effect of Δ cJun expression on inhibition of adenylyl cyclase activity by MOR or KOR (Figure 3). Two-way ANOVA of DAMGO concentration-effect curves showed a significant main effect of DAMGO concentration ($p < 0.0001$, $F = 20.26$, $df = 6$), but not of Δ cJun status ($p = 0.840$, $F = 0.041$, $df = 1$) and there was no significant interaction ($p = 0.982$, $F = 0.176$, $df = 6$). Similarly, there was no significant difference in $E_{\max}$ or EC_{50} values between mice with Δ cJun on ($E_{\max} = 23.6 \pm 2.6\%$; $EC_{50} = 304 \pm 43$ nM) or Δ cJun off ($E_{\max} = 26.1 \pm 2.5\%$, $p = 0.508$; $EC_{50} = 611 \pm 176$ nM, $p = 0.129$). Similar results were seen with U50,488, such that two-way ANOVA of the concentration-effect curves showed a significant effect of concentration ($p < 0.0001$, $F = 11.94$, $df = 6$), but not of Δ cJun status ($p = 0.127$, $F = 2.391$, $df = 1$) and there was no significant interaction ($p = 0.978$, $F = 0.190$, $df = 6$). Likewise, there were no significant differences in $E_{\max}$ or EC_{50} values between mice with Δ cJun on ($E_{\max} = 14.8 \pm 2.9\%$; $EC_{50} = 211 \pm 81$ nM) or off ($E_{\max} = 16.7 \pm 1.8\%$, $p = 0.597$; $EC_{50} = 360 \pm 151$ nM, $p = 0.411$).

Δ cJun expression also did not significantly affect inhibition of adenylyl cyclase in the NAc by the cannabinoid agonist. Two-way ANOVA of the WIN55,212-2 concentration-effect curves showed a significant main effect of WIN55,212-2 concentration ($p < 0.0001$, $F = 15.53$, $df = 6$), but not of genotype ($p = 0.066$, $F = 3.472$, $df = 1$) and there was no significant interaction ($p = 0.973$, $F = 0.208$, $df = 6$). Likewise, there were no significant differences in WIN55,212-2 $E_{\max}$ values ($13.0 \pm 2.3\%$ and $13.6 \pm 0.9\%$ inhibition in Δ cJun on versus off mice, respectively, $p = 0.821$) and or EC_{50} values (208 ± 120 nM and 417 ± 130 nM in Δ cJun on versus off mice, respectively, $p = 0.270$). Thus, although there was a slight trend toward decreased potency of WIN55,212-2 in mice expressing Δ cJun, the transgene did not significantly alter cannabinoid inhibition of adenylyl cyclase. Moreover, there was no effect of Δ cJun status on basal or forskolin-stimulated adenylyl cyclase activity. Basal adenylyl cyclase activity was 1095 ± 71 pmol/mg/min and 1007 ± 77 pmol/

mg/min ($p = 0.403$) in mice with $\Delta cJun$ on or off, respectively. Adenylyl cyclase activity stimulated by 1 μM forskolin was 4185 ± 293 pmol/mg/min versus 4032 ± 273 pmol/mg/min ($p = 0.706$) in mice with $\Delta cJun$ on or off, respectively.

3.4. Discussion

The results of this study revealed enhanced MOR-mediated G-protein activation and inhibition of adenylyl cyclase in the NAc of mice with inducible transgenic expression of $\Delta FosB$ in dynorphin/ D_1R containing neurons. KOR-mediated inhibition of adenylyl cyclase activity was also enhanced in the NAc of $\Delta FosB$ expressing mice, suggesting that $\Delta FosB$ regulates the endogenous opioid system in the NAc. The DAMGO E_{max} value was greater for MOR-stimulated [^{35}S]GTP γ S binding, and its EC_{50} value was lower for adenylyl cyclase inhibition, in $\Delta FosB$ over-expressing mice compared to control mice. These findings suggest the possibility of receptor reserve for effector modulation but not G-protein activation under the assay conditions examined. The finding that maximal inhibition of adenylyl cyclase by the KOR agonist was affected by $\Delta FosB$ expression suggests low receptor reserve for the KOR-mediated response, consistent with the low levels of KOR binding sites in mouse brain (Unterwald, et al., 1991). In contrast, CB_1R -mediated G-protein activity and inhibition of adenylyl cyclase were unaffected by $\Delta FosB$ expression, suggesting that the opioid and cannabinoid systems differ in their response to $\Delta FosB$ in these NAc neurons.

The effect of $\Delta FosB$ on opioid receptor-mediated signaling is consistent with our previous report that $\Delta FosB$ expression in the striatum altered acute and chronic effects of morphine (Zachariou, et al., 2006). One finding of that study was that mice with transgenic expression of $\Delta FosB$ in dynorphin/ D_1R striatal neurons were more sensitive to morphine in place conditioning than controls. Furthermore, this effect was mimicked by virally mediated expression of $\Delta FosB$ by site-specific injection into the NAc. These observations are consistent with the current results that show enhanced MOR signaling in the NAc.

We previously identified the gene encoding dynorphin as a target of $\Delta FosB$, and proposed that reduced dynorphin would be consistent with enhanced rewarding properties of morphine in $\Delta FosB$ bitransgenic mice (Zachariou, et al., 2006). The present results show that KOR-mediated inhibition of adenylyl cyclase in the NAc is enhanced in $\Delta FosB$ expressing mice, which might reflect a compensatory increase in KOR sensitivity following reduced dynorphin. Previous studies have shown that KOR was upregulated in certain brain regions of prodynorphin knockout mice, including NAc (Clarke, et al., 2003).

In contrast to $\Delta FosB$, inducible transgenic expression of $\Delta cJun$, the dominant negative truncated mutant of the $\Delta FosB$ binding partner cJun, did not alter adenylyl cyclase inhibition by MOR or KOR agonists. These results suggest that basal levels of $\Delta FosB$ expression, which are relatively low, do not play a significant role in maintaining opioid receptor signaling at this level of signal transduction in the NAc. The fact that the conditioned rewarding effect of morphine was decreased by $\Delta cJun$ expression in our previous study (Zachariou, et al., 2006) suggests either that morphine induction of $\Delta FosB$ during the conditioning procedure is important in regulating behavioral responses to the drug or that transcriptional effects of $\Delta FosB$ other than those affecting proximal signaling by opioid receptors might influence opioid reward. In any event, results of the present study show clearly that, when $\Delta FosB$ expression is elevated above basal levels in striatal dynorphin/ D_1R -expressing neurons, there is a robust increase in the coupling of MOR and KOR to inhibition of adenylyl cyclase in the NAc.

The mechanisms by which MOR- and KOR-mediated signaling are enhanced by $\Delta FosB$ overexpression are unclear, but we have previously shown that MOR levels, assessed by [3H]naloxone binding, are not different in the NAc of $\Delta FosB$ on versus off mice (Zachariou,

et al., 2006). The same study found that $G\alpha_1$ and 2 protein levels were not affected in this region by Δ FosB expression. However, previous gene expression array analyses showed that $G\alpha_o$ mRNA was upregulated in NAc of Δ FosB on mice (McClung and Nestler, 2003). It will be of interest in future studies to comprehensively examine the effect of transgenic Δ FosB expression on G-protein subunit expression at the protein level as well as on the expression of many G-protein modulatory proteins.

It is interesting that Δ FosB expression did not enhance CB_1R -mediated signaling in the NAc. It is possible that alterations in CB_1R signaling occur in a discrete population of neurons that is obscured in the whole NAc preparation. For example, administration of Δ^9 -THC significantly induced Δ FosB in the core, but not shell, of the NAc (Perrotti, et al., 2008). Indeed, it has been shown that challenge with Δ^9 -THC following repeated administration of Δ^9 -THC increased dopamine release in the NAc core, but decreased release in the shell (Cadoni, et al., 2008). It is also important to note that the 11A line of bitransgenic mice express Δ FosB only in dynorphin/ D_1R positive medium spiny neurons of the striatum, but CB_1R are expressed in both dynorphin/ D_1R and enkephalin/ D_2R positive striatal neurons (Hohmann and Herkenham, 2000), as well as on terminals of cortical afferents (Robbe, et al., 2001). Expression of the dominant negative regulator of Δ FosB-mediated transcription, Δ cJun, also had no significant effect on cannabinoid receptor signaling, although Δ cJun is inducibly expressed in both D_1 and D_2 -containing populations of medium spiny neurons in these mice (Peakman, et al., 2003). It is possible, however, that basal Δ FosB expression is sufficiently low that Δ cJun would not affect receptor signaling, as suggested by results with MOR and KOR. It is also possible that CB_1R signaling is modestly enhanced by basal Δ FosB expression, such that further increasing Δ FosB expression or blocking its actions with Δ cJun had only slight effects that did not reach the level of statistical significance. Indirect support for this interpretation can be seen by comparing WIN55,212-2 EC_{50} values between mice expressing Δ cJun versus Δ FosB. The ratio of the WIN55,212-2 EC_{50} value for adenylyl cyclase inhibition in mice with induced expression of Δ cJun to its EC_{50} value for G-protein activation in mice with induced expression of Δ FosB was 4.0, whereas the same ratio in mice without induction of either transgene was 1.2.

Alternatively, cannabinoids might induce Δ FosB expression without any direct effect on CB_1R signaling. In this scenario, cannabinoids could modulate responsiveness to the psychoactive effects of other drugs via Δ FosB-mediated transcriptional regulation. In fact, administration of Δ^9 -THC produces cross-sensitization to opioids and amphetamine (Cadoni, et al., 2001, Lamarque, et al., 2001), consistent with this hypothesis. Moreover, repeated administration of the cannabinoid agonist CP55,940 was reported to increase MOR-mediated G-protein activation in the NAc, similarly to mice inducibly expressing Δ FosB in the present study (Vigano, et al., 2005). The effect of Δ FosB expression on Δ^9 -THC-mediated behaviors has not been evaluated, but the present results do not preclude an interaction. The results of this and our previous study (Zachariou, et al., 2006) show Δ FosB-induced alterations in MOR and KOR/dynorphin in the striatum. The rewarding effects of Δ^9 -THC, as measured by place preference, are abolished in MOR null mice, whereas deletion of KOR attenuated Δ^9 -THC place aversion and revealed Δ^9 -THC place preference (Ghozland, et al., 2002). Similarly, conditioned place aversion to Δ^9 -THC is absent in pro-dynorphin knockout compared to wild-type mice (Zimmer, et al., 2001). These data suggest that Δ^9 -THC might be more rewarding after Δ FosB induction and consequent induction of MOR signaling with reductions in dynorphin expression.

In summary, the results of this study showed that expression of Δ FosB in D_1R /dynorphin positive striatal neurons enhanced MOR- and KOR-mediated signaling at the level of G-protein mediated inhibition of adenylyl cyclase activity in the NAc. This finding is

consistent with studies that have demonstrated a role for the endogenous opioid system in reward (Trigo, et al., 2010), and provide a potential mechanism for Δ FosB-mediated effects on reward. In contrast, CB₁R-mediated signaling in the NAc was not significantly affected by striatal Δ FosB expression under the conditions examined, although further studies are warranted to determine the effect of Δ FosB induction on the endocannabinoid system.

Research Highlights

- MOR signaling is enhanced in the nucleus accumbens of mice that express Δ FosB
- KOR inhibition of adenylyl cyclase is also enhanced in mice expressing Δ FosB
- Expression of Δ FosB does not alter CB₁R signaling in the nucleus accumbens

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Effect of Δ FosB expression on agonist-stimulated [35 S]GTP γ S binding in the NAc. Membranes from Δ FosB-expressing (Δ FosB on) or control (Δ FosB off) mice were assayed as described in Methods using varying concentrations of DAMGO (A) or WIN55,212-2 (B). Data are mean percent stimulation values $\pm$ SEM. There was a significant main effect of Δ FosB expression revealed by two-way ANOVA on G-protein activation by DAMGO ($p < 0.001$) but not WIN55,212-2 ($p = 0.172$).

Figure 2.

Effect of Δ FosB expression on inhibition of adenylyl cyclase activity in the NAc. Membranes from Δ FosB-expressing (Δ FosB on) or control (Δ FosB off) mice were assayed as described in Methods in the presence of 1 μ M forskolin with and without varying concentrations of DAMGO (A), U50,488H (B) or WIN55,212-2 (C). Data are mean percentage of inhibition of net forskolin-stimulated adenylyl cyclase values $\pm$ SEM. There was a main effect of Δ FosB expression revealed by two-way ANOVA on adenylyl cyclase inhibition by DAMGO ($p = 0.0012$) and U50,488H ($p = 0.0006$), but not WIN55,212-2 ($p = 0.735$).

Figure 3.

Effect of Δ cJun expression on inhibition of adenylyl cyclase activity in the NAc. Membranes from Δ cJun-expressing (Δ cJun on) or control (Δ cJun off) mice were incubated in the presence of DAMGO (A), U50,488H (B) or WIN55,212-2 (C) as described in Methods. Data are mean percentage of inhibition of net forskolin-stimulated adenylyl cyclase values $\pm$ SEM. There was no significant main effect of Δ cJun expression determined by two-way ANOVA on adenylyl cyclase inhibition by DAMGO ($p = 0.84$), U50,488H ($p = 0.127$) or WIN55,212-2 ($p = 0.066$).