A Single Dose of Methamphetamine Leads to a Long Term Reversal of the Blunted Dopamine D1 Receptor-mediated Neocortical c-fos Responses in Mice Deficient for D2 and D3 Receptors*

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Received for publication, June 12, 2000, and in revised form, September 25, 2000
Published, JBC Papers in Press, September 27, 2000, DOI 10.1074/jbc.M005064200

Dopamine D1 receptors play an essential role in the induction of expression of the immediate-early gene c-fos in response to pharmacological stimuli. In the forebrain of wild-type mice, administration of a D1 receptor agonist leads to c-fos mRNA expression levels that are substantially higher than corresponding levels expressed after indirect stimulation of dopamine receptors with methamphetamine. In mice deficient for D2 and D3 receptors, c-fos mRNA levels expressed in response to D1 agonist administration are significantly blunted. However, a single dose of methamphetamine (5 mg/kg) leads to a long lasting reversal of the blunted c-fos responses in these mutants. In the forebrain, this reversal is restricted to the neocortex. Moreover, methamphetamine also enhances c-fos expression levels in preadolescent wild-type mice that normally express low c-fos mRNA in response to D1 agonist stimulation. Thus, a single dose of methamphetamine leads to a long term increase in D1 receptor-dependent c-fos responses in brains with either low (preadolescent mice) or blunted (adult D2 and D3 mutant mice) c-fos expression levels. A similar long term reversal of the blunted c-fos responses is achieved with a single dose of a full D1 agonist. These results indicate that the constitutive inactivation of D2 and D3 receptors leads to a decrease in agonist-promoted D1 receptor activity that can be reversed by intermittent agonist stimulation.

The induction of expression of the immediate-early gene c-fos, a gene with low base-line levels of expression in brain, is a well established and powerful tool for examining neuronal circuits that are activated biochemically in response to a variety of different stimuli. For example, studies on the induction of c-fos in response to an acute administration of drugs of abuse identified a common neuroanatomical pattern of expression (for review see Ref. 1), and it has been shown that the subchronic administration of such drugs (which is associated with a progressive sensitization of neuronal systems) leads to distinct alterations in the anatomic pattern of c-fos expression (2). In addition, studies on mutant mice have demonstrated that the expression of dopamine D1 receptors is essential for the control of immediate-early gene expression by psychomotor stimulants, such as cocaine and amphetamine (3). Although D1 receptors are essential for the induction of c-fos expression in response to psychostimulants, the magnitude of the D1-dependent c-fos expression levels appears to be modulated by both D2 and D3 receptors. For example, the study of Moratalla et al. (3) identified anatomically restricted alterations in c-fos responses to haloperidol, a neuroleptic drug that blocks the D2-like dopamine receptor subtypes D2 and D3, and other studies on mice deficient for D3 receptors revealed blunted c-fos responses to D1 agonist stimulation. These responses were even further reduced when D3 mutants were pretreated with the D2-like antagonist eticlopride (4).

To further investigate the role of D2 and D3 receptors in the modulation of c-fos responses to pharmacological stimuli, the present study used mice deficient for D2 and D3 receptors to analyze their levels of forebrain c-fos mRNA expressed in response to (a) direct stimulation of D1 receptors with a full D1 agonist, (b) indirect stimulation of dopamine receptors via methamphetamine-induced dopamine release, and (c) agonist stimulation of D1 receptors 1–3 weeks after an application of either methamphetamine or the D1 agonist. This study revealed that the constitutive inactivation of D2 and D3 receptors leads to a decrease in agonist-promoted D1 receptor activity that can be reversed in a long term manner by a single dose of either methamphetamine or a D1 agonist.

MATERIALS AND METHODS

Animals—The generation of D2 and D3 receptor knockout mice was described previously (5). The present study used the fifth generation of congenic C57Bl/6 mutants and their wild-type littermates. Experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Columbia University. All mice were housed in groups of 4–5 animals/cage with free access to food and water. Animals housed in the same cage received the same drug treatment (see below) and were returned to their home cage after drug injection until they were killed by decapitation.

Drug Treatments—All drugs were dissolved in saline and administered intraperitoneally. The D1 agonist SKF82958, the D1 antagonist SCH23390, and S(-)-methamphetamine hydrochloride were purchased from Research Biochemicals, Inc. (Natick, MA). The doses of methamphetamine (2–5 mg/kg) administered to the animals were calculated based on the molecular weight of the salt compound (C_{10}H_{15}N.HCl).

RNA Extraction and Northern Blotting—After decapitation, the brain was rapidly removed, and the forebrain was dissected. For this dissection, the mesodiencephalic junction was used as the anatomic landmark for the caudal border of the forebrain. In some experiments, the forebrain neocortex was further dissected from the extraneocortical structures containing the striatum, hypothalamus, thalamus, and epithalamus. RNA was extracted using the guanidinium/cesium chloride ultracentrifugation method. 20 μg of total RNA (extracted from tissues pooled from 2 to 4 animals/genotype) was loaded onto each lane of the...
Northern blotting experiments (each performed with RNAs pooled from
two animals/genotype). For each series of these experiments, Northern
blots were probed with equal aliquots of the same 32P-radiolabeled c-fos
cDNA and exposed to the same film. Multiple means of optical densities
were compared with a one-way analysis of variance (ANOVA), and the
significance of differences was assessed by Duncan's Studentized Range
Test for comparisons of multiple means (threshold of significance, p <
0.05).

RESULTS

A first series of experiments determined the basal, D1 agonist,
and methamphetamine-induced c-fos mRNA levels in the
forebrains of D2 and D3 mutant mice and their wild-type
littermates. Compared with wild-type mice (in which c-fos
mRNA levels are undetectable), the basal c-fos mRNA levels
are higher in the forebrains of both D2 and D3 mutant mice (Fig. 1A).

The induction of c-fos mRNA expression was determined 60
min after application of the full D1 agonist SKF82958 (1 mg/
kg). This induction is robust in wild type, but by comparison it
is drastically blunted in both D2 and D3 mutants (Fig. 1A). A
comparison of optical densities (OD) determined for equal size
fields of the autoradiogram shown in Fig. 1A (OD wild type, 6.0;
D2 mutants, 0.5; D3 mutants, 0.8) indicates that the c-fos re-
sponses of D2 and D3 mutants are reduced to 8.3 and 13.3%,
respectively, of the corresponding wild-type level. In five inde-
pendent experiments (see “Materials and Methods”), c-fos
mRNA levels expressed in D2 mutants were only 12.2 ± 3.7% of
the corresponding wild-type levels (p < 0.001), and c-fos mRNA
levels of D3 mutants reached only 15.7 ± 9.4% of levels ex-
pressed in wild type (p < 0.001). c-fos mRNA levels expressed
in D2 and D3 mutants did not differ significantly (Table 1).

Another series of experiments measured c-fos mRNA levels
expressed in response to a single dose of methamphetamine (8 mg/
kg). In the forebrain of wild-type mice, c-fos responses are
substantially lower than corresponding responses to the D1
agonist (Fig. 1A). A comparison of optical densities on the
autoradiogram shown in Fig. 1A indicates that the magnitude of
these c-fos responses (OD, 1.0) is only 16.7% of the responses
detected in wild-type animals after D1 agonist application (OD,
6.0). In four independent experiments, c-fos levels expressed in
methamphetamine-treated wild-type mice reached only 23.7 ±
12.0% of the corresponding levels expressed in SKF-treated
wild-type mice (Student's t test, p < 0.001). c-fos mRNA levels
induced with only 2 mg/kg methamphetamine also do not differ
from c-fos mRNA levels induced with 8 mg/kg methamphetamine
(data not shown). Moreover, in contrast to the results
obtained with the D1 agonist, methamphetamine-induced c-fos
responses of D2 or D3 mutant mice do not differ from wild type
(Fig. 1A). Nevertheless, as shown in Fig. 1B in wild type and D2
and D3 mutants, c-fos responses to methamphetamine treat-
ment are markedly reduced by pretreatment with the D1 re-
cptor antagonist SCH23390 (0.3 mg/kg), but they are unaf-
fected by a pretreatment with saline. These data confirm that
the induction of c-fos expression by amphetamine-like drugs is
dependent upon D1 receptor activation (3).

Additional studies compared c-fos responses to D1 agonist
stimulation in drug-naive mice and mice that received a single
dose of methamphetamine (5 mg/kg) 1, 2, or 3 weeks before
SKF treatment. In wild-type animals, the c-fos responses to D1
agonist stimulation do not significantly alter 1 and 2 weeks
after methamphetamine pretreatment (Fig. 1C). Interestingly
however, as many as 2 weeks after a single dose of metham-
phetamine, the levels of c-fos induced in both D2 and D3 mu-
nants are similar to the levels expressed in either drug-naive or
methamphetamine-pretreated wild-type mice (Fig. 1C). Thus,
in contrast to the blunted c-fos responses to D1 agonist stimu-
lization of drug-naive mutants (Fig. 1A), methamphetamine-

1 The abbreviations used are: Pn, postnatal day n; OD, optical den-
sity; MAP, mitogen-activated protein.
pretreated D₂ and D₃ mutants show substantially more robust c-fos responses to D₁ agonist stimulation (see below). However, 3 weeks after methamphetamine administration, the blunted c-fos responses of the mutants to D₁ agonist stimulation are again apparent, and a comparison of the optical densities of signals on the autoradiogram shown in Fig. 1D (OD wild type, 4.08; D₂ mutants, 0.8; D₃ mutants, 1.0) indicates that D₂ and D₃ mutants express only 19.5 and 24.4% of the corresponding wild-type c-fos levels (these levels are similar to the levels determined above for SKF-treated drug-naive mutants).

The results summarized above were obtained from adult mice that received the first drug injection at P60. To determine whether similar results were obtained in preadolescent mice, experiments were also performed in mice at postnatal age 30. As shown in Fig. 2A, P30 mice express only marginally increased levels of c-fos in response to the D₁ agonist (1 mg/kg), and these levels do not differ from corresponding levels induced by methamphetamine (8 mg/kg). Moreover, at these low expression levels, no significant differences are found between wild type and D₂ mutants, and D₃ mutants express only slightly reduced levels of c-fos in response to both SKF and methamphetamine (Fig. 2A, see lanes marked 1, 2, and 3). However, when P30 mice (both wild type and mutants) are treated with a single dose of methamphetamine (5 mg/kg) and challenged with SKF at P47, c-fos responses are robust in all genotypes, and no differences are found between wild-type and mutant mice. Furthermore, D₂ single mutants that received methamphetamine at postnatal day 24 also express high levels of c-fos in response to SKF administered at P30, and the c-fos mRNA levels of these mutants do not differ from the levels expressed in mice treated with methamphetamine at P30 and challenged with SKF at P47 (Fig. 2B). Altogether, these results indicate that a single dose of methamphetamine leads to a long term increase in c-fos responses to D₁ agonist stimulation in brains with either low (preadolescent mice) or blunted (adult D₂ and D₃ mutant mice) c-fos expression levels.

To further determine whether the D₂ agonist and methamphetamine induce different levels of c-fos in different anatomic areas of the forebrain, additional experiments compared c-fos mRNA levels in the neocortex and remaining forebrain of adult wild-type and mutant mice. These results revealed that the levels of c-fos mRNA expressed in response to a single dose of methamphetamine are higher in the forebrain neocortex compared with the inner (extraneocortical) mass of the forebrain (Fig. 3). This finding is in contrast to the D₁ agonist, which induces similar levels of c-fos in the neocortex and in extraneocortical forebrain structures (Fig. 4). Moreover, in both D₂ and D₃ mutants that were pretreated with methamphetamine, drastically enhanced c-fos responses to SKF treatment are detected in the neocortex, but the c-fos mRNA levels in the extraneocortical forebrain remain low (Fig. 3). In fact, on the autoradiogram shown in Fig. 3, neocortical c-fos levels of D₂ and D₃ mutants are 3.4- and 2.1-fold higher, respectively, compared with wild-type c-fos levels (OD wild type, 1.37; D₂ mutants, 4.70; D₃ mutants, 2.87). By comparison, the optical densities of c-fos signals obtained from extraneocortical forebrain mRNA are 1.56, 0.5, and 0.5 for wild type, D₂ mutants, and D₃ mutants, respectively. Thus, the methamphetamine-induced reversal of the blunted c-fos expression of both mutants in

### Table 1

| Treatment | Brain region | Wild type ODs | D₂/−/− ODs | D₃/−/− ODs |
|-----------|--------------|---------------|-------------|-------------|
| SKF       | Forebrain    | 5 ± 1.5       | 0.6 ± 0.2*  | 0.7 ± 0.2*  |
| METH/SKF  | Neocortex    | 2.1 ± 0.5     | 3.9 ± 1.7   | 3.9 ± 1.7   |
| SKF/SKF   | Neocortex    | 0.9 ± 0.1     | 1.8 ± 0.2*  | 1.3 ± 0.1*  |

* p < 0.001 compared with wild type.
* p < 0.01 compared to wild type.
* p < 0.05 compared with wild type.
* p < 0.001 compared with D₂ mutants.  

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**FIG. 2.** c-fos mRNA responses to a D₁ agonist and methamphetamine in the forebrains of wild-type, D₂, and D₃ mutant mice at postnatal age P30. A, the c-fos mRNA levels expressed in P30 wild type (lanes 1), homozygous D₂ mutants (lanes 2), and homozygous D₃ mutants (lanes 3) in response to SKF82958 (SKF, 1 mg/kg) and methamphetamine (METH, 8 mg/kg) are compared with c-fos mRNA levels expressed in SKF-treated D₂ mutants at P60 (lane P60). B, c-fos mRNA levels of SKF-treated P30 wild-type mice (wt P30) are compared with corresponding mRNA levels expressed in wild-type (wt), homozygous D₂, and homozygous D₃ mutant mice that received a single injection of methamphetamine (5 mg/kg) at P30 followed by a single dose of SKF (1 mg/kg) at P47, and D₂ mutants that received an equal dose of methamphetamine at P15 followed by SKF treatment at P30. For each genotype, brain tissues of four animals/genotype were pooled for RNA extraction, and 20 μg of total RNA was loaded onto each lane. The blots shown in A and B were exposed to film for 16 and 6 h, respectively.

**FIG. 3.** c-fos mRNA responses to methamphetamine and SKF82958 in the neocortex and extraneocortical forebrain structures of wild-type, D₂, and D₃ mutant mice. Top left, c-fos mRNA levels detected in the neocortex (CTX) and extraneocortical forebrain (fb) 60 min after methamphetamine (8 mg/kg) treatment of P60 wild-type (wt) mice. Top middle, the blot shown on the left was reprobed with radiolabeled N-encoded cDNA. Top right, comparison of c-fos mRNA levels expressed in response to SKF treatment in the neocortex and in extraneocortical forebrains of wild type, homozygous D₂ (D₂), and D₃ (D₃) mutants that were treated with a single dose of methamphetamine (5 mg/kg) 1 week before SKF administration. Bottom, the first three lanes show basal levels of c-fos mRNA detected 1 week after the administration of a single dose of methamphetamine. The following lanes show c-fos responses to methamphetamine (8 mg/kg) given to either methamphetamine-pretreated animals (Meth/Meth) or drug-naive animals (Meth-naive). 20 μg of total RNA was loaded onto each lane, and all blots were exposed to film for 6 h.
A further comparison between c-fos responses of SKF-naive and SKF-pretreated animals revealed that SKF pretreatment, similar to methamphetamine pretreatment, markedly increased c-fos responses only in the mutants (Fig. 4, bottom). However, in contrast to methamphetamine, SKF does not significantly alter the increased basal levels of c-fos in D2 mutants. Also, D3 mutants continue to express detectable c-fos mRNA, although to a lesser extent (Fig. 4, bottom).

**DISCUSSION**

This study shows that compared with methamphetamine, higher c-fos mRNA levels are expressed in response to D1 agonist stimulation and that adult mice lacking either the D2 or D3 receptor show blunted c-fos responses to the D1 agonist. A single dose of methamphetamine induces a long lasting enhancement of c-fos responses in brains with either low (preadolescent wild-type and mutant mice) or blunted (adult D2 and D3 mutants) c-fos expression levels. Moreover, the enhanced c-fos responses to the D1 agonist seen in methamphetamine-pretreated adult mutants are only detected in the neocortex, a brain region in which the acute administration of methamphetamine itself induces the largest c-fos responses. Furthermore, a single dose of a full D1 agonist elicits similar long lasting enhancement of c-fos responses to subsequent D1 agonist stimulation. These data suggest that despite the unaltered expression of D1 ligand-binding sites in D2 and D3 mutants (8, 9), the chronic inactivation of D2 and D3 receptors leads to a decreased responsiveness of D1 receptors to agonist stimulation, which can be reversed by a single dose of either methamphetamine or a D1 agonist.

Significantly reduced c-fos protein responses to D1-agonist stimulation were previously reported (4) for the same D3 mutants in this study. The present analysis of c-fos mRNA expression levels revealed similarly blunted c-fos responses in mice deficient for D2 receptors, an effect that could only marginally be detected in the previous protein study (4). This analysis suggests that D2 but not D3 mutants develop compensatory mechanisms that operate either at the translational or post-translational level to maintain wild-type-like c-fos protein responses to D1 agonist stimulation. The present study also found increased basal levels of c-fos mRNA in the forebrain of mice deficient for D2 and D3 receptors. This result is similar to the results of previous pharmacological studies showing increased c-fos expression levels in rats that were treated acutely or chronically with the D2/D3 receptor blocker haloperidol (10, 11).

The c-fos responses to methamphetamine differ both quantitatively and qualitatively from the c-fos responses induced by the D1 agonist. In contrast to the widespread c-fos expression induced by the D1 agonist, the effects of methamphetamine are delimited to the neocortex. These effects suggest that the two types of pharmacological stimuli activate different neuronal populations/circuitries that express c-fos, and future and more detailed investigations of the anatomic distribution of c-fos mRNA expression will need to test investigation. The quantitative differences of c-fos expression levels induced by methamphetamine and D1 agonists may also reflect differences in the activation of the two principal transcriptional activators of the FOS gene, pMAP kinase and cAMP-response element-binding protein (pCREB) (12). In any case, a main finding of the present study is that a single dose of methamphetamine (5 mg/kg) leads to a long term (as many as 2 weeks) reversal of the blunted c-fos responses to D1 agonist stimulation in the forebrain of mice deficient for D2 and D3 receptors. Interestingly, the same dose of amphetamine has previously also been shown to induce a long-lasting behavioral and neuroendocrine sensitization in rats that is accompanied by an increase in electrically evoked dopamine release in the forebrain (13). Moreover,
as shown here one long term consequence of this dose of methamphetamine is a decrease of the (abnormally) high basal c-fos levels in D2 and D3 mutants. This consequence may perhaps be one of the mechanisms by which methamphetamine (but not the D1 agonist) increases the responsiveness of neurons to subsequent D1 agonist stimulation.

It will be of great interest to further elucidate the molecular mechanisms that lead to the methamphetamine-induced and D1 agonist-induced long term enhancement of cortical c-fos responses to D1 agonist stimulation in brains with low or abnormally blunted c-fos responses. The decreased agonist-promoted D1 receptor activity detected in D2 and D3 mutants suggest that the chronic treatment with neuroleptic drugs that block D2 and D3 receptors (a common therapeutic intervention of schizophrenia) could impair the function of D1 receptors. Several other studies provided evidence for a reduced cortical D1 receptor activity during chronic neuroleptic treatment (14–16), and results of a most recent study (14) suggested for the first time that a short term co-administration of a D1-selective agonist to monkeys chronically treated with neuroleptics can improve behavioral deficits associated with a decreased cortical D1 receptor activity. The present study now shows that the decreased response of neocortical D1 receptors to agonist stimulation in mice deficient for D2 and D3 receptors is not irreversible and suggests that an intermittent stimulation of dopamine release by amphetamine-like drugs during treatment with typical neuroleptics can result in a long term increase in agonist-promoted D1 receptor activity.

Acknowledgments—I thank Drs. James Howe and Hadassah Tamir for helpful comments on this manuscript.

REFERENCES
1. Harlan, R. E., and Garcia, M. M. (1998) Mol. Neurobiol. 16, 221–267
2. Curran, E. J., Akil, H., and Watson, S. J. (1996) Neurochem. Res. 21, 1425–1435
3. Moratalla, R., Xu, M., Tonegawa, S., and Graybiel, A. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14928–14933
4. Jung, M.-Y., and Schmauss, C. (1999) J. Biol. Chem. 274, 29406–29412
5. Jung, M.-Y., Skryabin, B. V., Arai, M., Abbondanzo, S., Fu, D., Brosius, J., Robakis, N. K., Polites, H. G., Pintar, J. E., and Schmauss, C. (1999) Neuroscience 91, 911–924
6. Van Beveren, C., van Straaten, F., Curran, T., Muller, R., and Verma, I. M. (1983) Cell 32, 1241–1255
7. Schmauss, C., McAllister, G., Ohosone, Y., Hardin, J. A., and Lerner, M. R. (1989) Nucleic Acids Res. 17, 1733–1744
8. Baik, J.-H., Picetti, R., Dalardi, A., Thiriet, G., Depaulis, A., Le Meur, M., and Borelli, E. (1995) Nature 377, 424–428
9. Xu, M., Koeltzow, T. E., Santiago, G. T., Moratalla, R., Cooper, D. C., Hu, X.-T., White, N. M., Graybiel, A. M., White, F. J., and Tonegawa, S. (1999) Neuron 19, 837–848
10. Robertson, G. S., and Fibiger, H. C. (1992) Neuroscience 46, 315–328
11. Merchant, K. M., Dobie, D. J., Filloux, F. M., Totke, M., Aravagiri, M., and Dorsa, D. M. (1994) J. Pharmacol. Exp. Ther. 271, 460–471
12. Fields, R. D., Rahete, F., Stevens, B., and Itoh, K. (1997) J. Neurosci. 17, 7253–7266
13. Vanderschuren, L. M. J., Schmidt, E. D., De Vries, T. J., Van Moorsel, C. A. P., Tilders, F. J. H., and Schoffelmeer, A. N. M. (1999) J. Neurosci. 19, 9579–9586
14. Castner, S. A., Williams, G. V., and Goldman-Rakic, P. S. (2000) Science 287, 2020–2022
15. Williams, G. V., and Goldman-Rakic, P. S. (1995) Nature 376, 572–575
16. Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., Someya, Y., Sassa, T., Sudo, Y., Matsushima, K., Iyo, M., Tateno, Y., and Turo, M. (1997) Nature 385, 634–638
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J. Biol. Chem. 2000, 275:38944-38948.
doi: 10.1074/jbc.M005064200 originally published online September 27, 2000

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