The Full D₁ Dopamine Receptor Agonist SKF-82958 Induces Neuropeptide mRNA in the Normosensitive Striatum of Rats: Regulation of D₁/D₂ Interactions by Muscarinic Receptors

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ABSTRACT
Neuropeptide and immediate early gene expression in striatonigral neurons of the normosensitive striatum is induced by mixed D₁/D₂ receptor agonists and indirect dopamine agonists, such as cocaine and amphetamine. Both D₁ and D₂ receptor antagonists block these events. In contrast, the partial D₁ agonist, SKF-38393, does not evoke striatonigral gene expression in intact rats. These findings have contributed to the idea that both D₁ and D₂ receptors must be stimulated to evoke gene expression in striatonigral neurons. How these “D₁/D₂ interactions” are accomplished is unclear in light of the controversy over whether striatonigral neurons express both D₁ and D₂ receptors. This study addresses these issues by demonstrating that in intact rats 1) a full D₁ receptor agonist, SKF-82958, induced behavioral activity and preprodynorphin (PPD) and substance P (SP) gene expression in medium spiny neurons in the dorsal, and especially, in the ventral striatum, 2) either a D₁ antagonist, SCH-23390, or a D₂ antagonist, eticlopride, blocked these effects, 3) the muscarinic antagonist, scopolamine, augmented PPD and SP mRNA expression induced by SKF-82958 and prevented the ability of eticlopride to block SKF-82958-induced PPD and SP mRNAs and 4) the SKF-82958-induced increase in preproenkephalin mRNA in striatopallidal neurons was blocked by SCH-23390 or scopolamine but not by eticlopride. These data indicate that endogenous acetylcholine attenuates D₁ receptor-stimulated PPD/SP gene expression in medium spiny neurons, mediates D₂ receptor-stimulated preproenkephalin gene expression in striatopallidal neurons and contributes to D₂ receptor involvement in D₁-stimulated PPD/SP gene expression.

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ABBREVIATIONS: PPD, preprodynorphin; SP, substance P; PPE, preproenkephalin; AUC, area under curve; CPU, caudoputamen; NAc, nucleus accumbens.
Recent studies have provided evidence that cholinergic interneurons are in a strategic position to mediate D1/D2 interaction transynaptically. First, D1 agonists increase, whereas D2 agonists decrease, acetylcholine release (Ajima et al., 1990; Bertorelli and Consolo, 1990; Consolo et al., 1993; Damsma et al., 1990, 1991). Second, muscarinic receptor stimulation suppresses PPD and SP mRNA in striatonigral neurons (Lucas and Harlan, 1995; Wang and McGinty, 1996c). Blockade of muscarinic receptors increases basal, and potentiates D1-stimulated, PPD and SP mRNA levels in the intact striatum (Wang and McGinty, 1996c). Therefore, the increase in SP/PPD mRNA induced by selective D1 agonists may be attenuated by acetylcholine release. Furthermore, by decreasing acetylcholine release, D2 receptor activation would remove a brake on SP/PPD gene expression and thus enable striatonigral neurons to fully respond to D1 receptor stimulation. In contrast, muscarinic agonists stimulate PPE mRNA expression in striatopallidal neurons whereas muscarinic antagonists block psychostimulant-induced increases in PPE mRNA (Lucas and Harlan, 1995; Wang and McGinty, 1996c). Therefore, by increasing acetylcholine release, psychostimulants may be able to increase PPE mRNA induction transsynaptically.

In this study, quantitative in situ hybridization was used to examine the contribution of muscarinic receptors to the transsynaptic regulation of striatal gene expression induced by D1 receptor activation. Experiment I investigated whether acute injection of the full D1 agonist, SKF-82958, would induce PPD, SP and PPE mRNA expression in the intact rat striatum. Experiment II investigated whether D1 and D2 receptor antagonists would block SKF-82958-stimulated gene expression. Once these questions were answered positively, experiment III investigated whether 1) the muscarinic receptor antagonist, scopolamine, would augment SKF-82958-stimulated PPD and SP, but block PPE gene induction in the striatum and 2) whether scopolamine would prevent the ability of the selective D2 antagonist, eticlopride, to block SKF-82958-induced gene expression.

**Materials and Methods**

**Animals.** Adult male Wistar rats (240–270 g, Charles River, Raleigh, NC) were individually housed and maintained on a 12-hr light/dark schedule with food and water provided ad libitum. All animals were handled on a daily basis for at least 2 days before the experiment to minimize stress. On the day of the experiment, the animals received injections and were observed in the quiet home circle (diameter 1.2 mm rostral to bregma (Paxinos and Watson, 1986).

**Experimental protocols.** Three experiments were carried out in this study. Experiment I examined dose-dependent effects of SKF-82958 (RBI, Natick, MA) on striatal neuropeptide expression. Rats were randomly divided into five groups (n = four per group). Each rat received one injection of saline or one dose of SKF-82958 (0.02, 0.1, 0.5 and 2 mg/kg). Experiment II evaluated the contribution of D1 and D2 dopamine receptors to SKF-82958-stimulated gene expression. The effects of pharmacological blockade of D1 receptors by SCH-23390 (0.1 mg/kg, RBI) or blockade of D2 receptors by eticlopride (0.5 mg/kg, RBI) on SKF-82958- (2 mg/kg) stimulated neuropeptide mRNA expression were investigated in 6 groups (n = 4 per group): saline + saline, SCH-23390 + saline, eticlopride + saline, saline + SKF-82958, SCH-23390 + SKF-82958, eticlopride + SKF-82958. Experiment III was designed to explore whether muscarinic receptors mediated either or both findings from the second experiment: 1) that eticlopride blocked SKF-82958-stimulated PPD and SP gene expression and 2) that SKF-82958 stimulated PPE expression. In this experiment, following pretreatment with saline (four groups, n = four per group) or the nonselective muscarinic antagonist, scopolamine (5 mg/kg) (four groups, n = four per group), rats received injections either of saline + saline, eticlopride (0.5 mg/kg) + saline, saline + SKF-82958 (0.5 mg/kg) or eticlopride (0.5 mg/kg) + SKF-82958 (0.5 mg/kg).

All drugs were freshly prepared in physiological saline and injected s.c. in a volume of 1.2 ml/kg. The interval between injections in experiments II and III was 15 min. The behavior of the rats was rated by two trained raters, who were unaware of the treatment, 5 min before the first injection, every 5 min during the first hour and every 15 min for the next 2 hr after the final injection. Ratings were determined using a nine-point scale modified from Ellinwood and Balster (1974): 1) asleep, inactive; 2) normal activity, grooming; 3) increased activity; 4) hyperactive running with jerky movement; 5) slow patterned movement (repetitive exploration); 6) fast patterned movement (repetitive exploration with hyperactivity); 7) stereotypy (repetitive sniffing/rearing in one location to the exclusion of other activities); 8) continuous gnawing, sniffing or licking and 9) dyskinesia, seizures.

**In situ hybridization histochemistry.** Three hours after a single injection (experiment I) or the final injection (experiments II and III), the rats were anesthetized with equithesin (5 ml/kg, i.p.) and decapitated. A 3-hr time point was chosen because peak induction of striatal PPD (Wang et al., 1995), PPE (Bannon et al., 1989) and SP (Bannon et al., 1991; Haverstick et al., 1989) mRNA expression occurs 3 hr after dopamine stimulation by amphetamine. The brains were removed and frozen in isopentane at −40°C and stored at −70°C. Quantitative in situ hybridization histochemistry to test PPD, SP and PPE mRNA expression in striatal neurons was performed according to standard procedures in this laboratory (Wang et al., 1995; Wang and McGinty, 1995a, b).

Quantitation of the mRNA hybridization signals on x-ray films was performed using NIH Image 1.44 (W. Rasband, NIMH) and a Macintosh Iici as detailed in our previous reports (Wang and McGinty, 1995b). Briefly, the 14C standards were measured, plotted against known dpm/mg, and converted to 35S equivalents to generate a calibration curve. Film background was measured and saved as a “blank field” to correct uneven illumination. Hybridization signals were measured under the density slice option. The upper limit was set to eliminate any background and this value was used to measure all images. The lower limit was set at the bottom of the LUT scale. The use of density slicing allows specific analysis of heterogeneous induction patterns in brain regions (such as patches in the caudate in which the hybridization signal is above that in the matrix). The hybridization signal in the caudoputamen was measured using a circle (diameter = 200 pixels). The signals in the NAc were measured using a manual drawing that outlined the shell or core areas. Quantitative changes were expressed as 1) the number of labeled pixels per area (area), 2) mean density of tissue in dpm/mg and 3) integrated density which is the product of area times mean density. As previously described (Wang and McGinty, 1995b), the integrated density measurement takes into account not just the average intensity of the signal, but the entire area over which the signal is expressed above threshold. We have found this value to be more representative of changes in hybridization signal than mean density alone. The mean ± S.E.M. of each of these measures was calculated for each rat by averaging the values in four adjacent sections at AP 1.2 mm rostral to bregma (Paxinos and Watson, 1986).

**Statistics.** A one-way analysis of variance followed by a Bonferroni (Dunn) comparison of groups using least squares-adjusted means was performed on the AUC values calculated from plotting behavioral ratings against time (Smith and McGinty, 1994). Significance in area, mean density and integrated density between groups was determined by a nested two-way analysis of variance followed by
Results

Behavior. Behavioral activity ratings from all three experiments are summarized in figure 1. In experiment I, SKF-82958 increased behavioral activity in a dose-dependent manner (fig. 1A). At 0.1 and 0.5 mg/kg, but not 0.02 mg/kg, SKF-82958 significantly increased locomotor activity (sniffing, grooming, rearing and locomotion). At the highest dose of 2 mg/kg, SKF-82958 initially induced hyperlocomotion, which included hanging on the bars of the cage top and jumping. These behaviors were usually replaced by multiple stereotypical behaviors (continuous sniffing, exploration or rearing in one place) within 25 to 30 min.

In experiment II, the rats were pretreated with either SCH-23390 (0.1 mg/kg) or eticlopride (0.5 mg/kg) before SKF-82958. It was found that SKF-82958 (2 mg/kg) no longer produced any visible enhancement of locomotor activity in the presence of either SCH-23390 or eticlopride (fig. 1B). Behavioral rating scores after administration of either antagonist were not significantly different from those in saline controls.

Behavioral responses to the different drug treatments in experiment III are illustrated in figure 1C and D. Note that the dose of SKF-82958 in this experiment was 0.5 mg/kg. In rats pretreated with saline (fig. 1C), behavioral changes resembled those observed in experiment II (fig. 1B) except that the intensity and duration of behaviors induced by SKF-82958 was proportional to the lower dose. Scopolamine (5 mg/kg, fig. 1D) by itself induced significant locomotor activity and SKF-82958, behavioral activity was not significantly different than that displayed by rats treated with scopolamine plus saline or scopolamine plus eticlopride.

Experiment I. Effects of SKF-82958 on striatal neuropeptide mRNA expression. Dose-dependent induction of PPD, SP and PPE mRNAs was seen in the striatum 3 hr after acute injection of SKF-82958 in doses above 0.02 mg/kg (fig. 2). The alterations in integrated density reflect a change in the mean density of the hybridization signal and, to a much larger extent, the number of labeled particles per area (data not shown). At the lowest dose (0.02 mg/kg), SKF-82958 had no effect on any of the mRNA levels in any region of the striatum. Reliable increases in all three mRNA hybridization signals were detectable after 0.1 mg/kg and were robust after 0.5 or 2 mg/kg. The PPD induction in the dorsal striatum (fig. 2A) was characteristically patchy (not shown) whereas the PPD induction in the ventral striatum, including shell and core regions of the NAc and the olfactory tubercle, was more homogeneous and much greater, especially in the shell area, than that in the dorsal striatum. SP induction (fig. 2B), unlike the pattern of PPD mRNA, occurred in a more homogeneous pattern throughout the dorsal striatum with greatest intensity in the lateral caudoputamen. In the ventral striatum, extremely strong induction of SP mRNA occurred in the shell area of the NAc and throughout the olfactory tubercle and islands of Calleja. However, SP mRNA expression in the core area of the NAc was not altered by SKF-82958 administration at any dose (fig. 2B). Induction of the PPE hybridization signal in the dorsal striatum was also homogeneous but no change was detectable in the ventral striatum (fig. 2C).

Experiment II. Effects of D1 and D2 receptor blockade on SKF-82958-stimulated striatal neuropeptide gene expression. Pretreatment with the D1 receptor antagonist, SCH-23390 (0.1 mg/kg), significantly reduced basal

## Fig. 1. Behavioral activity ratings illustrating dose-dependent effects of SKF-82958 (0.02, 0.1, 0.5 and 2 mg/kg, s.c.) on basal behavioral activity (A) and effects of D1 and D2 receptor blockade by SCH-23390 (0.1 mg/kg, s.c.) and eticlopride (0.5 mg/kg, s.c.), respectively, on SKF-82958-stimulated behaviors (B). In addition, effects of scopolamine (5 mg/kg, s.c.) on SKF-82958 (0.5 mg/kg, s.c.) stimulated behaviors and eticlopride (0.5 mg/kg, s.c.) induced blockade of SKF-82958-stimulated behavior are shown in C and D. The behavioral ratings are expressed as mean ± S.E.M. (n = four in each group). The numbers in the top-right legend of each panel represent the AUC values.

*P < .01 as compared with saline (A), saline + saline (B) or saline + saline + saline (C and D). *P < .01 as compared with saline + SKF-82958 (B) or saline + saline + SKF-82958 (C), *P < .01 as compared with saline + eticlopride + saline, *P < .01 as compared with saline + saline + SKF-82958 and scopolamine + saline + saline, *P < .01 as compared with saline + saline + SKF-82958.
Experiment III. Effects of muscarinic receptor blockade on SKF-82958-stimulated neuropeptide gene expression in the presence or absence of eticlopride.

In this experiment, the increase in PPD and SP expression induced by 0.5 mg/kg SKF-82958 was blocked by 0.5 mg/kg eticlopride (figs. 5B vs. C and G vs. H and 6). In the rats pretreated with 5 mg/kg scopolamine, PPD and SP induction in response to saline + SKF-82958 was substantially augmented in the caudoputamen and the shell areas of the NAc as compared to that in rats pretreated with saline (figs. 5B vs. D and G vs. 1 and 6). However, scopolamine had no significant effect on SKF-82958-stimulated PPD mRNA in the core area of the NAc (fig. 6B). In the presence of scopolamine, eticlopride did not alter SKF-82958-stimulated PPD (figs. 5D vs. E and 6A and B) or SP (figs. 5I vs. J and 6C and D) expression. In fact, the SKF-82958-stimulated PPD and SP mRNAs were augmented to the same extent by scopolamine in the presence or absence of eticlopride. In addition, in rats treated with scopolamine + saline + saline, a moderate increase in the basal levels of PPD and SP in both dorsal and ventral striatum was exhibited as compared to that in the saline + saline + saline group (fig. 6).

PPPE mRNA was increased by eticlopride (fig. 7B and F) or SKF-82958 (fig. 7C and F) whereas scopolamine treatment by itself did not affect the basal level of PPE expression in the striatum (fig. 7F). However, scopolamine partially blocked eticlopride-induced (fig. 7B vs. D and F), and completely blocked SKF-82958-stimulated (fig. 7C vs. E and F) PPE expression in the dorsal striatum.

Discussion

A series of experiments was conducted in this study to characterize alterations in neuropeptide gene expression in specific populations of striatal neurons after direct D1 dopamine receptor stimulation in intact rats. The major findings include the following. First, the full D1 receptor agonist, SKF-82958, unlike the partial D1 agonist, SKF-38393, was a potent stimulator of behavior as well as PPD/SP mRNA in striatonigral neurons and PPE mRNA in striatopallidal neurons in the normosensitive striatum. SKF-82958-stimulated PPD/SP expression was displayed in distinct patterns within the striatum; stronger induction was concentrated in the NAc than in the caudoputamen. This pattern closely parallels the stronger induction of c-fos and zif/268 expression in nucleus accumbens than in caudoputamen elicited by this drug (Wang and McGinty, 1996b). However, the pattern is unique because all other direct or indirect dopamine agonists induce greater gene expression in the caudoputamen than in nucleus accumbens. Second, D1 and D2 receptors cooperatively mediated the stimulating effects of SKF-82958 on behavior and gene expression as demonstrated by the equal sensitivity of SKF-82958-stimulated changes to D1 and D2 blockade. Third, scopolamine augmented SKF-82958-stimulated behavior and PPD/SP induction whereas it blocked SKF-82958-stimulated PPE induction, supporting the idea that striatal acetylcholine inhibits gene expression in striatonigral neurons and facilitates gene expression in striatopallidal neurons (Di Chiara et al., 1994; Wang and McGinty, 1996b,c,d). Finally, reduction of cholinergic transmission by systemic scopolamine prevented eticlopride from blocking SKF-82958-stimulated PPD/SP induction in rats that still
had elevated motor activity. These data suggest that muscarinic cholinergic receptors contribute to the ability of D₂ antagonists to block D₁-stimulated gene expression.

The full D₁ receptor agonist, SKF-82958, unlike the partial D₁ receptor agonist, SKF-38393, stimulates behavior and gene expression in intact animals. In intact rats, SKF-82958 strongly stimulates behaviors (Meyer and Shults, 1993; Murray and Waddington, 1989; this study), immediate early gene (Wang and McGinty, 1996b) and neuropeptide (this study) gene expression whereas SKF-38393, commonly regarded as the prototypical D₁ agonist, does not (Gerfen et al., 1990; Jiang et al., 1990; La Hoste et al., 1993; Paul et al., 1992; Robertson et al., 1991). Differences in the actions of these two structurally related benzazepine derivatives reflect, in part, the fact that SKF-82958 more powerfully stimulates D₁-coupled adenylate cyclase. SKF-38393, the first compound shown to have a selective action at the D₁ receptor (Setler et al., 1978), has only 45 to 70% of the maximum efficacy of dopamine in stimulating adenylate cyclase that is much less than that of SKF-82958 (149% intrinsic activity of dopamine) as demonstrated in homogenates of rat striatum (Anderson and Jansen, 1990; O'Boyle et al., 1989). Furthermore, SKF-38393 has limited ability to penetrate the blood brain barrier in contrast to SKF-82958 that is more lipophilic (Pfeiffer et al., 1982). These properties may contribute to the reason why SKF-82958, but not SKF-38393, possesses the power to stimulate behavioral and gene expression in intact rats. Further evidence supporting the importance of the efficacy of D₁ agonists in stimulating adenylate cyclase is provided by the observation that the full D₁ agonist, A-77636 with 134% intrinsic activity (Kebabian et al., 1992), induces Fos immunoreactivity in the dorsal striatum of intact rats (Wirtshafter and Asin, 1994).

**Is the D₂ receptor contribution to SKF-82958-stimulated PPD and SP gene expression in striatonigral neurons mediated by acetylcholine?** The PPD/SP induc-
tion by SKF-82958 is a D₁-mediated event because blockade of D₁ receptors by SCH-23390 prevented the induction. However, the PPD/SP induction is also mediated by D₂ receptors because D₂ receptor blockade by eticlopride significantly attenuated the increases. How D₂ receptors regulate striatopallidal gene expression is puzzling given the controversy over colocalization of D₁/D₂ receptors in these neurons (Gerfen et al., 1990; Le Moine et al., 1990; Le Moine and Bloch, 1995; Surmeier et al., 1993) and the inhibitory effect of D₂ receptor stimulation on adenylate cyclase activity. Two major alternatives, which involve transynaptic mechanisms, exist: 1) direct synaptic connections between D₂-expressing striatopallidal neurons and D₁-expressing striatonigral neurons (Yung et al., 1996) and/or 2) indirect synaptic interactions that are mediated by interneurons. With regard to the former, because eticlopride causes an increase in striatal PPE (and GAD) (Soghomonian, 1994) mRNA and enkephalin immunoreactivity, it is assumed that striatopallidal neurons
are activated by D₂ receptor blockade. Such activation may lead to an increase in inhibition of striatonigral neurons via direct contacts that would result in a decreased ability of D₁ receptor stimulation to increase PPD/SP expression. Although this pathway may contribute to some types of D₁/D₂ interactions, its contribution to the ability of scopolamine to completely block eticlopride’s effects on SKF-stimulated SP/PPD gene expression is not obvious. Instead, this study and others (reviewed in Di Chiara et al., 1994 and Wang and McGinty, 1996d) suggest that cholinergic interneurons are involved in these D₁/D₂ interactions. We hypothesize that D₂ dopamine receptor stimulation reduces cholinergic inhibition of striatanginal gene expression by decreasing acetylcholine release (Ajima et al., 1990; Bertorelli and Consolo, 1990; Damsma et al., 1990). The result would be the same as blocking muscarinic receptors with scopolamine that enables striatonigral neurons to positively and fully respond to D₁ stimulation. In contrast, D₁ receptor blockade would stimulate acetylcholine release and the subsequent muscarinic receptor stimulation would considerably suppress the responsiveness of striatonigral neurons to D₁ receptor stimulation. A muscarinic antagonist would prevent the D₂ antagonist’s effect on striatanginal gene expression by blocking the effects of acetylcholine release. Moreover, in this study, muscarinic receptor blockade by scopolamine completely reversed eticlopride’s ability to block SKF-82958-stimulated PPD/SP induction.

Is the D₁-mediated increase in PPE mRNA in striatangidal neurons mediated by acetylcholine? In contrast to the negative regulation of striatanginal PPD/SP gene expression by striatal acetylcholine, striatangidal PPE gene expression is positively regulated by cholinergic neurotransmission. In our previous study, systemic injection of the muscarinic receptor agonist, oxotremorine, up-regulated PPE mRNA expression (Wang and McGinty, 1996c). In a parallel way, an increase in endogenous release of acetylcholine (Consolo et al., 1993; Damsma et al., 1991; Florin et al., 1992; Lindefors et al., 1992; Mandel et al., 1994) may contribute to an increase in PPE mRNA expression in response to amphetamine stimulation because systemic (Wang and McGinty, 1996c) or intrastriatal (Wang and McGinty, 1997) administration of scopolamine attenuated amphetamine induction of PPE mRNA. Furthermore, in this study, scopolamine abolished SKF-82958-stimulated PPE induction. It is noteworthy that direct D₁ receptor stimulation by SKF-82958 produced higher levels of PPE induction than did amphetamine administration (Wang and McGinty, 1996a). The greater effect of SKF-82958 on PPE mRNA expression is consistent with stronger stimulation of D₁ vs. D₂ receptors (in contrast to a more equal stimulation of D₁/D₂ receptors by amphetamine) that should result in a larger increase in acetylcholine release (Ajima et al., 1990; Bertorelli and Consolo, 1990; Damsma et al., 1990).
coupled to phosphoinositide hydrolysis (Hulme et al., 1990) and expressed by all striatopallidal neurons (Bernard et al., 1992; Weiner et al., 1990), may contribute to PPE induction by neuroleptics. This notion is supported by substantial evidence from this study and others that muscarinic receptor blockade attenuates neuroleptic-stimulated Fos (Guo et al., 1992) and enkephalin immunoreactivity (Hong et al., 1980, 1985) and PPE mRNA in the striatum (Angulo et al., 1990; Augood et al., 1992; 1993; Pollack and Wooten, 1992; this study).

Although eticlopride was not able to block SKF-82958-stimulated PPD/SP expression in the presence of scopolamine, eticlopride still significantly attenuated SKF-82958-stimulated behaviors after scopolamine pretreatment. This result may be due to residual enhancement of striatopallidal neuronal activity after eticlopride blockade of D₂ receptors that scopolamine only partially attenuates. Activated striatopallidal neurons would counteract the influence of the striatonigral pathway on behavioral activation, thus contributing to the locomotor depressant effects of neuroleptics.

**Is the striatum an important site for the dopamine/acetylcholine interactions in regulation of striatonigral and striatopallidal peptide gene expression?** Keefe and Gerfen (1995) established that intrastriatal microinfusion of D₂- or D₃-selective antagonists decreases the ability of SKF-38393 and quinpirole coadministration to induce immediate early gene expression in the striatum of 6-OHDA-lesioned rats. Regarding cholinergic regulation of striatal gene expression, Nisenbaum et al. (1994) reported that seven daily injections of scopolamine prevented the 6-hydroxydopamine lesion-induced elevation of PPE mRNA and reduction of SP mRNA in the striatum. However, seven daily intrastriatal injections of scopolamine in a concentration range of 0.5–50 mM were not able to mimic this prevention. In contrast, recent data from this laboratory (Wang and McGinty, 1997) indicate that intrastriatal injection of the muscarinic receptor antagonist, oxotremorine, at a concentration of 1.6–8.1 mM, inhibited PPD/SP mRNA induced by amphetamine and increased PPE mRNA expression. Intrastriatal injection of the muscarinic receptor antagonist, scopolamine (62 mM), increased basal levels of PPD/SP expression and augmented amphetamine-stimulated PPD/SP mRNA expression. Intrastriatal scopolamine also blocked amphetamine-stimulated PPE mRNA expression. Furthermore, amphetamine-induced behavioral activity was completely blocked by intrastriatal oxotremorine and augmented by intrastriatal scopolamine.
(Wang and McGinty, 1997). These data are in good accordance with those observed after acute systemic injection of oxotremorine and scopolamine (Wang and McGinty, 1996c). Thus, intrastratial dopamine/acetylcholine interactions contribute to the regulation of tonic and phasic striatal neuropeptide gene expression under normal and dopamine-stimulated conditions. However, the contribution of extrastriatal cholinergeric regulation of dopamine-dependent changes in striatal peptide gene expression should not be overlooked because it may preferentially function in specific pathophysiological processes and under different experimental conditions.

Based on available data (Di Chiara et al., 1994; Wang and McGinty, 1996d; this study), an hypothesized model of cell-to-cell interactions between dopamine and acetylcholine in the regulation of striatal peptide gene expression is summarized in figure 8. SKF-82958 increases PPD/SP expression by direct stimulation of D1 receptors on striatonigral and accumbal neurons and striatopallidal PPE expression indirectly by facilitating SP-induced (DeBoer and Abercrombie, 1994) release of acetylcholine or, possibly, by stimulating D2 receptors that are expressed by a subset of cholinergeric interneurons, at least in primates (Bergson et al., 1995). Etclopride increases PPE expression by direct and indirect mechanisms, i.e., blocking D2 receptors directly on striatopallidal neurons and increasing the facilitatory influence of acetylcholine on PPE expression by blocking D2 inhibition of acetylcholine release. The increased acetylcholine release evoked by eticlopride could also exercise its inhibitory influence on SKF-82958-stimulated PPD/SP expression. Scopolamine augments tonic and phasic PPD/SP induction most likely by blocking M1 receptors on striatonigral neurons and attenuates SKF-82958- or eticlopride-stimulated PPE mRNA induction most likely by blocking M4 receptors on striatopallidal neurons. Furthermore, scopolamine prevents the ability of eticlopride to decrease D1 receptor-stimulated PPD/SP expression by blocking the interaction of acetylcholine, released by eticlopride, with its receptors. Further studies will investigate the location and identification of the specific muscarinic receptor subtypes involved.

**Conclusions**

This study demonstrates that a full D1 receptor agonist induces neuropeptide mRNA expression in the normosensitive dorsal and ventral striatum. The induction of PPD/SP mRNAs in striatonigral neurons is prevented by D1 and D2 receptor blockade, indicating a participation of D2 receptor tone in the full expression of D1-stimulated gene expression. The induction of PPE mRNA in striatopallidal neurons is blocked by D1 and muscarinic receptor blockade, indicating that the D1-mediated induction of PPE involves transynaptic activation of cholinergeric neurotransmission. Finally, because eticlopride failed to prevent SKF-82958-stimulated striatonigral gene expression in the presence of scopolamine, D2 receptor blockade most likely prevents the stimulating effect of SKF-82958 by enhancing inhibitory cholinergeric tone on striatopallidal neurons.

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