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LOCAL INFUSION OF THE (±)-α-AMINO-3-HYDROXY-5-METHYLISOXAZOLE-4-PROPIONATE/KAINATE RECEPTOR ANTAGONIST 6-CYANO-7-NITROQUINOXALINE-2,3-DIONE DOES NOT BLOCK D_1 DOPAMINE RECEPTOR-MEDIATED INCREASES IN IMMEDIATE EARLY GENE EXPRESSION IN THE DOPAMINE-DEPLETED STRIATUM

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Abstract—Administration of selective agonists of D_1 dopamine receptors increases immediate early gene expression in striatal neurons, a response which is particularly robust in the dopamine-depleted striatum. Although interactions between dopamine and glutamate receptor-mediated responses in striatal neurons have been demonstrated in a number of experimental paradigms, our previous findings indicate that *N*-methyl-D-aspartate antagonists do not block D_1 receptor-mediated induction of immediate early genes in the dopamine-depleted striatum. In the present study, we therefore examined interactions between D_1 dopamine receptors and the (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate subtypes of glutamate receptor by determining whether striatal infusion of the (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione would block D_1 receptor-mediated induction of the immediate early genes c-*fos* and *zif268* in the dopamine-depleted striatum. Striatal infusion of 6-cyano-7-nitroquinoxaline-2,3-dione (1 mM) completely blocked (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate-induced c-*fos* and *zif268* expression. However, 6-cyano-7-nitroquinoxaline-2,3-dione (1 μM–1 mM) did not significantly affect induction of c-*fos* and *zif268* by D_1 receptor stimulation (SKF 38393, 2 mg/kg, i.p.) in the dopamine-depleted striatum. To more generally block excitatory input, tetrodotoxin (10 μM) was infused into the striatum of rats receiving a D_1 agonist. Local infusion of tetrodotoxin had minimal effect on induction of c-*fos* and *zif268* in the dopamine-depleted striatum. In contrast, tetrodotoxin abolished induction of c-*fos* and *zif268* messenger RNAs by the D_2 antagonist eticlopride (0.5 mg/kg, i.p.) in both intact rats and dopamine-depleted rats receiving continuous D_2 agonist treatment (quinpirole, 0.5 mg/kg/day).

The results indicate that D_1 receptor-mediated induction of immediate early genes in the dopamine-depleted striatum occurs by mechanisms that are independent of excitatory input through (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptors.

Key words: glutamate, c-*fos*, *zif268*, SKF 38393, *in situ* hybridization, tetrodotoxin.

Medium spiny neurons of the striatum receive excitatory afferents from the cortex and thalamus,[32,34,42] and dopamine input from the substantia nigra.[18] Most of the excitatory postsynaptic current seen in striatal efferents is carried through the (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA)/kainate subtypes of glutamate receptors, although *N*-methyl-D-aspartate (NMDA) receptors also contribute to excitatory postsynaptic currents under depolarizing conditions.[11,26,28] The dopamine input, on the other hand, is thought to modulate the response of striatal neurons to excitatory input.[10,35]

Striatal projection neurons consist of striatonigral and striatopallidal neurons, defined by their main axonal projections and the neuropeptides and dopamine receptors they express.[20,22,25,29,56] Stimulation of D_1 dopamine receptors, which are predominantly expressed by striatonigral neurons,[20,27,39,40] increases immediate early gene expression in striatonigral neurons of both intact[9,57,63] and dopamine-depleted rats.[21,51] In addition, stimulation of corticostriatal fibers or administration of glutamate receptor agonists induces Fos protein in the striatum.[2,5,19,38,49] In the intact striatum, the D_1 dopamine receptor-mediated induction of immediate early genes by

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate; chloro-APB, (±)-SKF 82958 hydrobromide; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EDTA, ethylenediaminetetra-acetate; i.s., intrastriatal; NMDA, *N*-methyl-D-aspartate; 6-OHDA, 6-hydroxydopamine; SSC, saline sodium citrate; TTX, tetrodotoxin.

indirect dopamine agonists such as amphetamine is blocked by systemic administration of antagonists of both NMDA and AMPA/kainate subtypes of glutamate receptors,[37,58,59] suggesting that these two neurotransmitter systems interact in regulating immediate early gene expression in striatal neurons. We have shown previously, however, that blockade of NMDA receptors does not block induction of the immediate early genes c-*fos* and *zif268* in the dopamine-depleted striatum by D_1 dopamine receptor stimulation.[31] The purpose of this study therefore was to examine whether D_1 dopamine receptor-induced immediate early gene expression in the dopamine-depleted striatum is dependent on excitatory input through AMPA/kainate receptors.

To accomplish this, the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was infused via a microdialysis probe into the striatum of unanesthetized rats that had unilateral depletions of striatal dopamine. In addition, to further evaluate the role of excitatory input in D_1 receptor-induced immediate early gene expression, we locally infused tetrodotoxin (TTX) into the striatum to more generally block afferent input. Rats with unilateral dopamine depletions were used to examine these interactions between dopamine and glutamate systems, because the dopamine-depleted striatum allows us to examine the effects of D_1 dopamine receptor stimulation in the absence of D_2 dopamine receptor activation, which can alter the immediate early gene response to D_1 receptor stimulation.[21,30,50]

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (Taconic, NY) weighing approximately 200 g were housed in groups of three in a temperature-controlled room on a 12-h : 12-h light–dark schedule. Rats had free access to food and water. All surgical procedures and experimental manipulations were approved by the Institutional Animal Care and Use Committee at the National Institute of Mental Health, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All attempts were made to minimize animal suffering and to reduce the number of animals used.

Materials

Sodium pentobarbital was obtained from Anpro Pharmaceutical (Arcadia, CA). 6-Hydroxydopamine hydrobromide (6-OHDA) and TTX (with citrate buffer) were obtained from Sigma Chemical Company (St Louis, MO). AMPA, CNQX, (±)-SKF 38393 hydrochloride, (±)-SKF 82958 hydrobromide (chloro-APB), quinpirole hydrochloride and eticlopride hydrochloride were obtained from Research Biochemicals International (Natick, MA). The doses of all drugs were calculated as the salt, with the exception of 6-OHDA and AMPA, which were calculated as the free base. CNQX was prepared for intrastriatal (i.s.) infusion by dissolving it at a concentration of 10 mM in 0.1 M NaOH. The CNQX was then diluted to the desired concentration in artificial cerebrospinal fluid (ACSF). TTX was dissolved in deionized water at a concentration of 1 mM and then diluted 1:100 in ACSF for i.s. administration. AMPA was dissolved in ACSF at the desired concentration. The pH of the resulting solutions was adjusted to 7.4–7.6 with 0.1 M HCl or 1 M NaOH.

Dopamine depletions

Rats were anesthetized with sodium pentobarbital (80–90 mg/kg, i.p.) and placed in a stereotaxic instrument (Kopf Instruments). A 26-gauge needle, connected to a 50-μl Hamilton syringe on a syringe pump (Model 2000; Instech Laboratories, Inc.), was lowered into the right medial forebrain bundle/rostral substantia nigra at the following coordinates relative to interaural zero with the skull flat (in mm): AP +3.5, ML +1.5, DV +2.0. Two microliters of 6-OHDA (16 μg, calculated as free base) in 0.02% ascorbic acid/0.9% NaCl were infused at a rate of 0.5 μl/min. The cannula was left in place for 3 min after the infusion.

Pharmacological manipulations

Local infusions of CNQX or TTX were made through a microdialysis probe implanted in the striatum. Three to four weeks after the injection of 6-OHDA, rats were anesthetized with sodium pentobarbital, and a concentric microdialysis probe, constructed as described previously,[1,13,53] was implanted in the right striatum at the following coordinates relative to bregma with the skull flat (mm): AP +0.5, ML 3.0, DV −7.0. The microdialysis probe incorporated a dialysis membrane with a molecular weight cut-off of 13,000 (Spectra/Por RC, Spectrum) and had an active dialysing length of 3.5 mm and an external diameter of 250 μm. During implantation, the probe was perfused at a rate of 2 μl/min with ACSF of the following composition (mM): NaCl 144, KCl 2.0, $MgSO_4$ 1.2, KH_2PO_4 0.4, $CaCl_2$ 1.2. The pH of the ACSF was adjusted to 7.3–7.6 with 0.1 M NaOH. Rats were returned to their home cage after the surgery and allowed to recover for at least 24 h before the experiments began. The dialysis probe was not perfused during this period.

On the day of the experiments, rats were placed in cylindrical Plexiglas cages. The dialysis probe was connected to a 3-ml syringe (Sherwood Monoject) on the infusion pump via a fluid swivel (Instech Laboratories, Inc.) and a tether of polyethylene tubing (PE-20, Clay Adams) to allow the animal to move freely in the cage during the i.s. infusion of drugs. To examine the effects of AMPA on immediate early gene expression in the striatum, the probe was perfused with ACSF for 15 min at a rate of 0.5 μl/min. The perfusion medium was then switched to ACSF containing AMPA (1 mM) for 20 min. At the end of the AMPA infusion period, the perfusion medium was changed back to plain ACSF. The animal was killed 40 min later. When investigating the ability of locally infused CNQX to block AMPA-mediated changes in immediate early gene expression, the dialysis probe was infused with CNQX (1 mM) for 15 min. The perfusion medium was then changed to one containing both AMPA (1 mM) and CNQX (1 mM) for 20 min. At the end of that infusion, the perfusion medium was returned to that containing CNQX alone, and the animals were killed 40 min later.

To determine the effects of local AMPA/kainate receptor blockade on D_1-mediated changes in immediate early gene expression, animals were prepared as outlined above. On the day of the experiment, the dialysis probe was connected to the fluid swivel and tether and perfused for 15 min with CNQX. The rat then received a systemic injection of the D_1 dopamine receptor agonist (±)-SKF 38393 (2 mg/kg in 0.02% ascorbic acid, 1 ml/kg, i.p.) and was killed 1 h later. Perfusion of the dialysis probe with CNQX continued during the i.p. injection and postinjection survival period. Control animals received i.s. infusion of ACSF and systemic injection of SKF 38393 (2 mg/kg, i.p.) or i.s. infusion of CNQX and systemic injection of ascorbic acid vehicle solution.

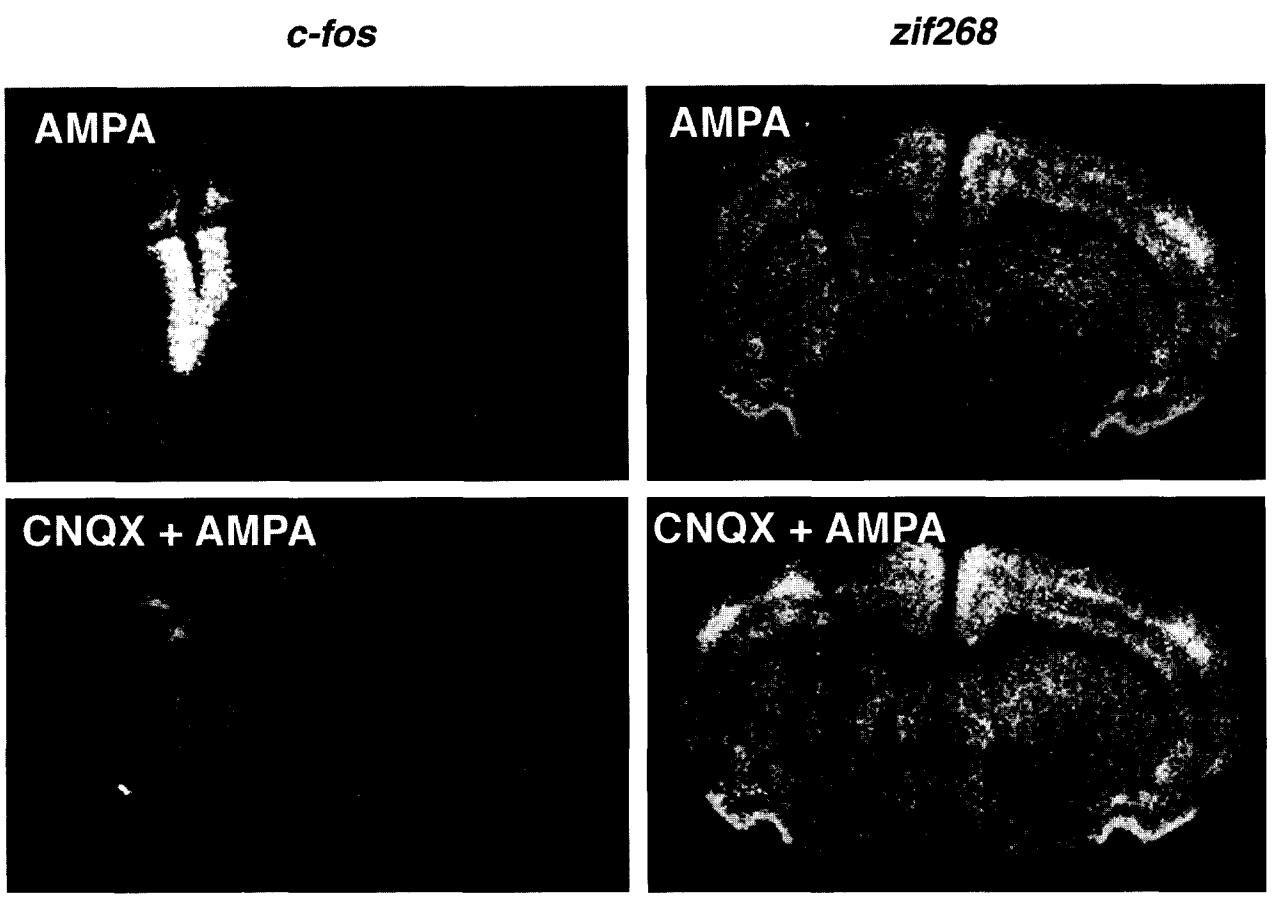

Fig. 1. *In situ* film autoradiograms showing the expression of c-*fos* and *zif268* in unanesthetized rats receiving i.s. perfusion of AMPA (1 mM) or co-infusion of CNQX and AMPA (both 1 mM) via a microdialysis probe implanted in the dopamine-depleted striatum. AMPA was infused into the striatum for 20 min. Rats were killed 40 min after the end of the AMPA infusion. To determine the ability of CNQX infused locally into the striatum to block AMPA/kainate receptors, CNQX infusion through the microdialysis probe began 15 min before the introduction of AMPA to the perfusion medium and continued throughout the 20-min exposure to AMPA and the 40-min survival period.

To examine the effect of TTX infusion into the striatum on dopamine-mediated changes in immediate early gene expression, the infusion of TTX was started when the animal was connected to the fluid swivel and continued throughout the duration of the experiment. One hour after the infusion of TTX began, a time at which neurotransmitter release in the striatum has been shown to be significantly decreased by TTX,[62] the rats were given an i.p. injection of a dopamine receptor agonist or antagonist. Rats were killed 1 h after the injection. Control rats received i.s. infusions of ACSF and/or systemic injections of the vehicle solution used to deliver the dopamine receptor agents. Some dopamine-depleted rats receiving TTX were also implanted with osmotic mini-pumps (Alzet, Model 2001, Alza Corp., CA) three to five days before the dialysis probe was implanted, to examine the effects of TTX on immediate early gene induction produced by blockade of D_2 dopamine receptors in the dopamine-depleted striatum. Control rats underwent sham surgical procedures to control for the effects of anesthesia. After the dialysis probe was implanted, all procedures were as outlined above.

In situ *hybridization*

For *in situ* determination of mRNA levels, rats were killed with CO_2 and decapitated. The brains were rapidly removed and frozen in isopentane (Fluka Chemicals, Ronkonkoma, NY) chilled on dry ice. Brains were cut in 12-μm sections on a cryostat (Frigocut 2800E, Cambridge Instruments GmbH, Germany). Sections were thaw-mounted on to gelatin/chrome–alum-subbed slides and stored at −70°C until processed further. Slides for all animals within a given experiment then were thawed at room temperature, postfixed in 4% formaldehyde/0.9% NaCl for 10 min, acetylated in fresh 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl (pH 8.0) for 10 min, dehydrated in an ascending series of alcohols, delipidated in chloroform, and then rehydrated in a descending series of alcohols. Slides were air dried and then stored at −20°C.

For detection of *zif268* and c-*fos* mRNAs, 48-base oligonucleotide probes complementary to bases 352–399 of *zif268* cDNA[43] and 1227–1274 of c-*fos* cDNA,[14] respectively, were synthesized on an Applied Biosystems DNA synthesizer. Purified probes were end-labeled with [^{35}S]dATP and terminal deoxynucleotidyl transferase (Boehringer Mannheim, Indianapolis, IN), and diluted in hybridization buffer to obtain 2×10^6 c.p.m./100 μl. The hybridization buffer contained: 0.6 M NaCl, 80.0 mM Tris (pH 7.5), 4.0 mM EDTA, 0.1% (w/v) sodium pyrophosphate, 10% (w/v) dextran sulfate, 0.2% (w/v) sodium dodecyl sulfate, 0.02% (w/v) heparin sulfate, 50% (v/v) formamide and 100 mM dithiothreitol. Ninety microliters of hybridization buffer with probe were applied to each slide containing three to four sections and covered with a glass coverslip.

Table 1. Effects of intrastriatal infusion of 6-cyano-7-nitroquinoxaline-2,3-dione on immediate early gene expression in the dopamine-depleted striatum

Treatment	*zif268*	c-*fos*
ACSF (i.s.)	10.2±1.3 (3)	2.3±0.2
CNQX (1 mM, i.s.)	10.3±2.0 (3)	3.6±0.6
ACSF (i.s.)	17.9±0.7 (4)	4.7±0.2
AMPA (1 mM, i.s.)	31.1±5.8 (6)	34.4±4.0*
CNQX (1 mM)+AMPA (1 mM, i.s.)	21.3±1.1 (7)	8.8±1.1†
NMDA (1 mM, i.s.)	39.1±2.0*(5)	22.1±1.6*
CNQX (1 mM)+NMDA (1 mM, i.s.)	43.7±4.7 (4)	27.6±8.9

CNQX or ACSF was infused into the striatum via a microdialysis probe for 75 min, beginning 15 min before the i.s. infusion of AMPA or NMDA was begun. Infusion of the agonist continued for 20 min, at which time the perfusion medium was switched back to that containing ACSF or CNQX alone. Rats were killed 40 min later and the brains processed for *in situ* hybridization histochemistry. Values are the mean grey values minus white matter labeling (arbitrary units; ±S.E.M.) measured from film autoradiograms of the striatum at the rostrocaudal level containing the track created by the microdialysis probe. Numbers in parentheses are the number of animals per treatment group. Note that the data presented are from two different experiments; thus, there are two different groups of rats receiving ACSF, i.s. *Significantly different from ACSF, $P<0.05$. †Significantly different from AMPA alone, $P<0.05$.

Slides were hybridized for 12–18 h in humid chambers at 37°C. Upon removal, slides were washed four times in 1 × SSC (0.15 M NaCl/0.015 M sodium citrate, pH 7.2) at room temperature and then three times for 20 min each in 2 × SSC with 50% (v/v) formamide at 40°C. Finally, slides were washed 2 × 30 min in 1 × SSC at room temperature, dipped in deionized water and air dried. Labeled slides were apposed to X-ray film (X-Omat, Eastman Kodak Co., Rochester, NY) for three days to one month.

Data analysis

Film autoradiograms were analysed using the image analysis program Image (Wayne Rasband, National Institutes of Health) to obtain average density (grey) values. Prior to the measurement of brain sections, the linearity of the video camera and video capture card to increasing signal intensity was determined by measuring the average value of signals of known optical density from a photographic step tablet. The intensity of the illuminating light was then adjusted so that the values from the film autoradiograms of brain sections fell within the linear portion of the system's response. The images of sections from all experimental and control groups within a given experiment that were processed and hybridized in parallel were captured and measured under constant lighting conditions. Measurements were taken over the entire dopamine-depleted striatum dorsal to the anterior commissure at the rostrocaudal level of the striatum containing the track created by the dialysis probe. The cannula track was excluded from analysis. The average grey value of the white matter overlying the striatum was subtracted from the average grey value of the striatum to correct for background labeling.

The effects of drug treatments on the expression of immediate early genes in the striatum, as indicated by changes in the average grey values, were analysed with a one-way analysis of variance followed by post hoc analysis with the Tukey–Kramer test. Significance was set at $P<0.05$.

RESULTS

Blockade of (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate-induced immediate early gene expression in the dopamine-depleted striatum by 6-cyano-7-nitroquinoxaline-2,3-dione

The local infusion of AMPA (1 mM) into the dopamine-depleted striatum via a microdialysis probe for 20 min resulted in significant induction of the immediate early gene c-*fos* in a U-shaped area around the cannula track (Fig. 1, Table 1). The local infusion of AMPA also produced an increase in the expression of the immediate early gene *zif268* in the striatum (Fig. 1, Table 1), although the increase in *zif268* expression was less pronounced than the induction of c-*fos* and did not reach statistical significance ($P=0.06$). To determine whether local administration of CNQX into the striatum effectively blocks AMPA/kainate receptors, CNQX (1 mM) was co-infused with AMPA into the striatum. The local infusion of CNQX into the striatum blocked the induction of c-*fos* by AMPA and reduced the expression of *zif268* (Fig. 1, Table 1). The local infusion of CNQX alone had no effect on the basal expression of either c-*fos* or *zif268*, nor did it significantly affect immediate early gene expression induced by intrastriatal infusion of 1 mM NMDA (Table 1).

Rats receiving an i.s. infusion of AMPA did not exhibit signs of seizure activity. In general, the rats began sniffing, grooming and turning contralaterally within 5 min of the onset of the AMPA infusion. This activity, along with vacuous chewing and some rearing, continued throughout the infusion of AMPA and the 40-min post-infusion survival period. Animals co-infused with CNQX and AMPA showed similar behaviors.

Effects of intrastriatal infusion of 6-cyano-7-nitroquinoxaline-2,3-dione on D_1 dopamine receptor-mediated increases in immediate early gene expression in the dopamine-depleted striatum

Administration of the D_1 dopamine receptor agonist SKF 38393 (2 mg/kg, i.p.) to rats with unilateral depletions of striatal dopamine, as has been reported previously,[30,51,52] significantly increased the expression of c-*fos* and *zif268* in the dopamine-depleted striatum of rats receiving an i.s. infusion of ACSF (Fig. 2). Intrastriatal infusion of the AMPA/kainate

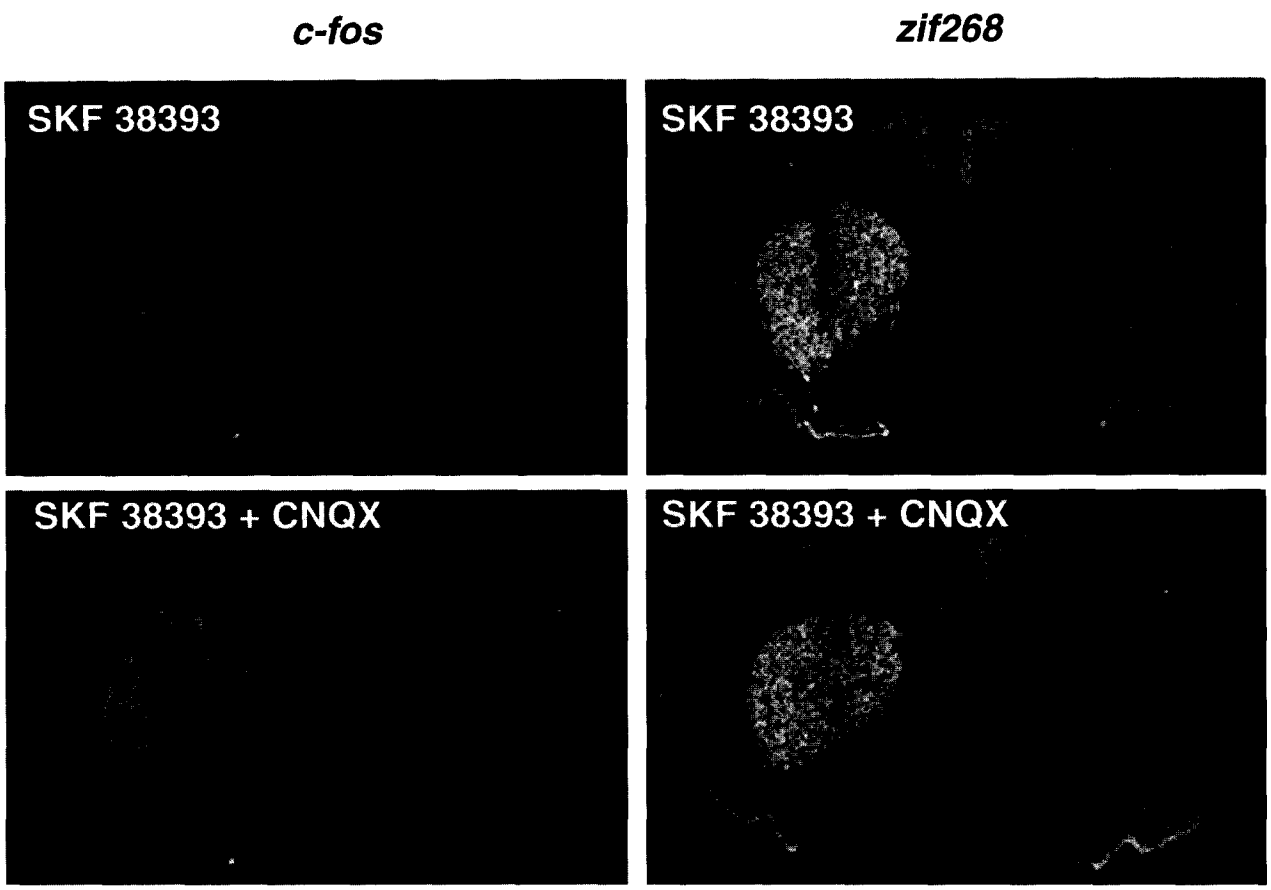

Fig. 2. *In situ* hybridization film autoradiograms showing the effects of i.s. infusion of the AMPA/kainate receptor antagonist CNQX (1 mM) via a microdialysis probe implanted in the dopamine-depleted striatum on the expression of c-*fos* and *zif268* induced by systemic administration of the D_1 dopamine receptor agonist SKF 38393 (2 mg/kg, i.p.). CNQX was dissolved in 0.1 M NaOH and then diluted in ACSF and perfused through the microdialysis probe, beginning 15 min before the systemic administration of SKF 38393 and continuing throughout the 60-min survival period. Control rats received an i.s. perfusion of ACSF and systemic administration of SKF 38393.

receptor antagonist CNQX at doses ranging from 10 μM to 1 mM had no significant effect on the D_1-mediated induction of either c-*fos* (Fig. 2, Table 2) or *zif268* (Fig. 2, Table 2; $P>0.05$), despite the fact that the 1 mM concentration effectively blocked the immediate early gene response to AMPA infusion. The i.s. infusion of CNQX also had no apparent effect on the contralateral rotation induced by systemic injection of the D_1 agonist, nor did it induce rotation when administered alone into the striatum (data not shown).

Effects of intrastriatal infusion of tetrodotoxin on D_1 dopamine receptor-mediated increases in immediate early gene expression in the dopamine-depleted striatum

To further evaluate the role of afferent activity in the D_1-mediated induction of immediate early genes, TTX was locally infused into the striatum. Local infusion of TTX had no significant effect on the basal expression of c-*fos* or *zif268* (Table 4). The infusion of 10 μM TTX into the dopamine-depleted striatum failed to significantly affect SKF 38393-induced expression of *zif268* ($P>0.05$; Fig. 3, Table 3), and produced only a small (23%), but statistically significant, attenuation of c-*fos* relative to the expression seen in the striatum of rats receiving a systemic injection of SKF 38393 and an i.s. infusion of ACSF.

Administration of chloro-APB (SKF 82958; 0.5 mg/kg, i.p.), a full D_1 dopamine receptor agonist, to rats with unilateral depletions of striatal dopamine also resulted in marked induction of *zif268* and c-*fos* in the dopamine-depleted striatum (Fig. 4, Table 3). Intrastriatal infusion of TTX did not affect the induction of either immediate early gene in the lesioned striatum by chloro-APB ($P>0.05$).

When TTX was infused into the dopamine-depleted striatum of rats with a unilateral lesion of the nigrostriatal pathway, the rats rotated contralateral to the striatum in which the TTX was infused. This contralateral rotation typically began within 10 min of the onset of the infusion of TTX and continued throughout the 2-h period of infusion,

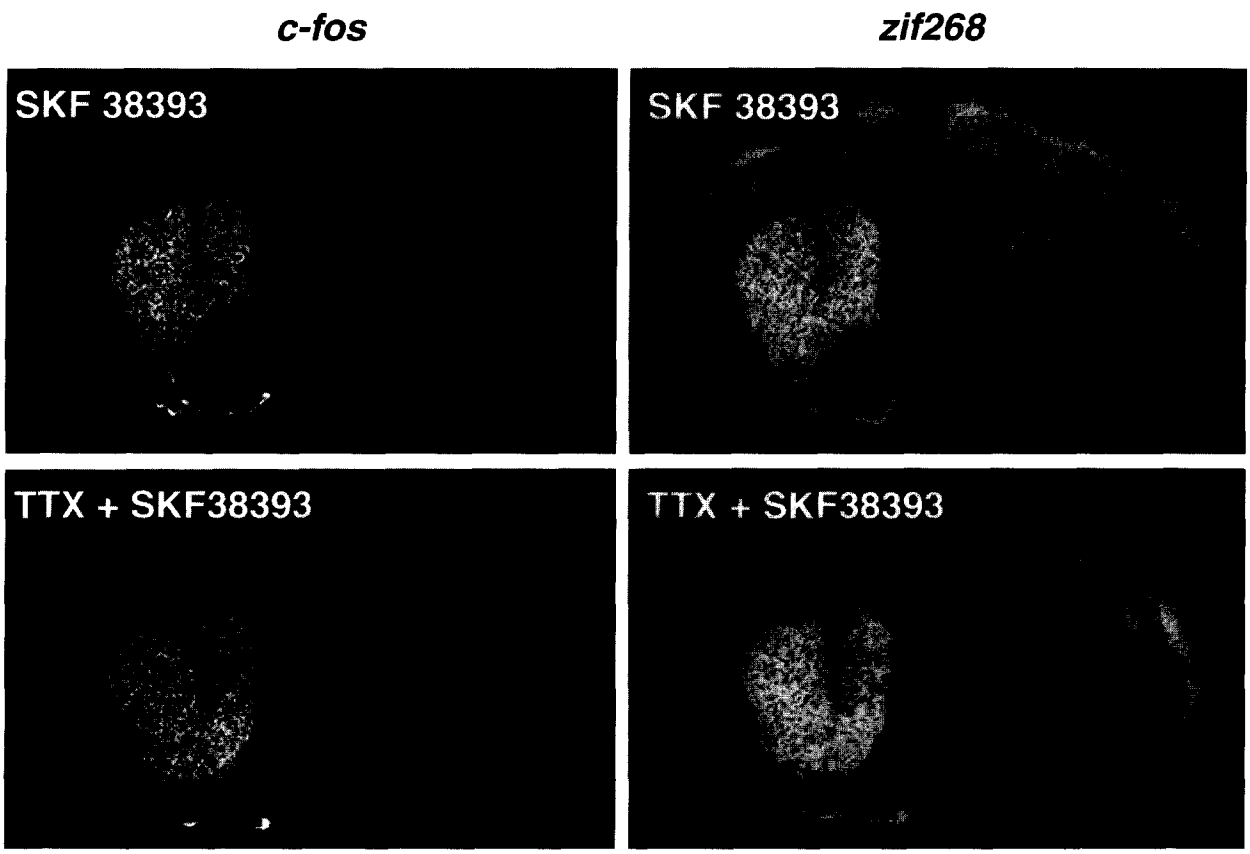

Fig. 3. *In situ* hybridization film autoradiogram showing the effects of i.s. infusion of TTX (10 μM) via a microdialysis probe implanted in the dopamine-depleted striatum on the expression of c-*fos* and *zif268* induced by systemic administration of the D_1 dopamine receptor agonist SKF 38393 (2 mg/kg, i.p.). TTX was dissolved in water and then diluted in ACSF and perfused through the microdialysis probe beginning 1 h before the systemic administration of SKF 38393 and continuing throughout the 60-min survival period. Control rats received an i.s. perfusion of ACSF and systemic administration of SKF 38393.

although the frequency of the rotation decreased over time. Systemic injection of SKF 38393 or chloro-APB also induced rotation contralateral to the dopamine-depleted striatum. The frequency of the rotation increased throughout the 1-h survival period. Intrastriatal infusion of TTX suppressed the contralateral rotation induced by SKF 38393 and chloro-APB.

Effects of intrastriatal infusion of tetrodotoxin on D_2 dopamine receptor-mediated increases in immediate early gene expression

The ability of CNQX to block AMPA-induced immediate early gene expression provided a positive control to demonstrate that CNQX was blocking AMPA/kainate receptors. However, with local infusion of TTX, direct measurement of blockade of voltage-sensitive sodium channels in the awake, freely-moving rat was beyond the scope of this project. Therefore, to provide a control for the ability of i.s. TTX to block dopamine-mediated changes in immediate early gene expression, we examined the effects of locally infused TTX on immediate early gene expression induced by manipulations of D_2 dopamine receptors. Systemic administration of the D_2 dopamine receptor antagonist eticlopride (0.5 mg/kg, i.p.) increased the expression of both *zif268* and c-*fos* in the striata of neurologically intact rats (Fig. 5, Table 4). Local infusion of 10 μM TTX into the striatum completely eliminated the induction of both *zif268* and c-*fos* in the infused striatum by the D_2 dopamine receptor antagonist (Fig. 5, Table 4). The induction of the immediate early genes by eticlopride in the non-infused striatum was not affected by the infusion of TTX into the contralateral striatum (Fig. 5).

Because it was possible that the differences in the effects of i.s. TTX on immediate early gene expression induced by the D_1 and D_2 dopamine receptor manipulations reflected differences between the dopamine-depleted and intact striatum, we also examined the effects of i.s. infusion of TTX on dopamine-depleted rats treated continuously with a D_2 dopamine receptor agonist. In rats with unilateral depletions of nigrostriatal dopamine that received continuous systemic administration of the D_2 dopamine receptor agonist quinpirole (0.5 mg/kg/day) for four to six days, systemic administration

Table 2. Effects of intrastriatal infusion of 6-cyano-7-nitroquinoxaline-2,3-dione on immediate early gene expression induced by systemic administration of the D_1 dopamine receptor agonist SKF 38393

Treatment	*zif268*	c-*fos*
ACSF (i.s.)+vehicle (i.p.)	10.2 ± 1.3 (3)	2.3 ± 0.2
ACSF (i.s.)+SKF 38393 (2 mg/kg, i.p.)	68.7 ± 2.0*(6)	33.1 ± 1.7*
10 μM CNQX (i.s.)+SKF 38393 (i.p.)	69.2 ± 2.4*(4)	34.8 ± 3.0*
100 μM CNQX (i.s.)+SKF 38393 (i.p.)	70.1 ± 2.0*(5)	34.2 ± 4.1*
1 mM CNQX (i.s.)+SKF 38393 (i.p.)	66.1 ± 1.7*(5)	27.2 ± 5.4*

CNQX or ACSF was infused into the striatum via a microdialysis probe for 75 min, beginning 15 min before the administration of SKF 38393 (2 mg/kg, i.p.) to rats with unilateral 6-OHDA-induced depletion of the nigrostriatal dopamine pathway. Rats were killed 1 h after the injection of the D_1 agonist or the vehicle solution and the brains processed for *in situ* hybridization histochemistry. Values are the mean grey values minus white matter labeling (arbitrary units; ± S.E.M.) measured from film autoradiograms of the striatum at the rostrocaudal level containing the track created by the microdialysis probe. Numbers in parentheses are the number of animals per treatment group. *Significantly different from ACSF (i.s.)+vehicle (i.p.), $P<0.05$. None of the groups treated with CNQX were statistically different from the ACSF (i.s.)+SKF 38393 (2 mg/kg, i.p.) group.

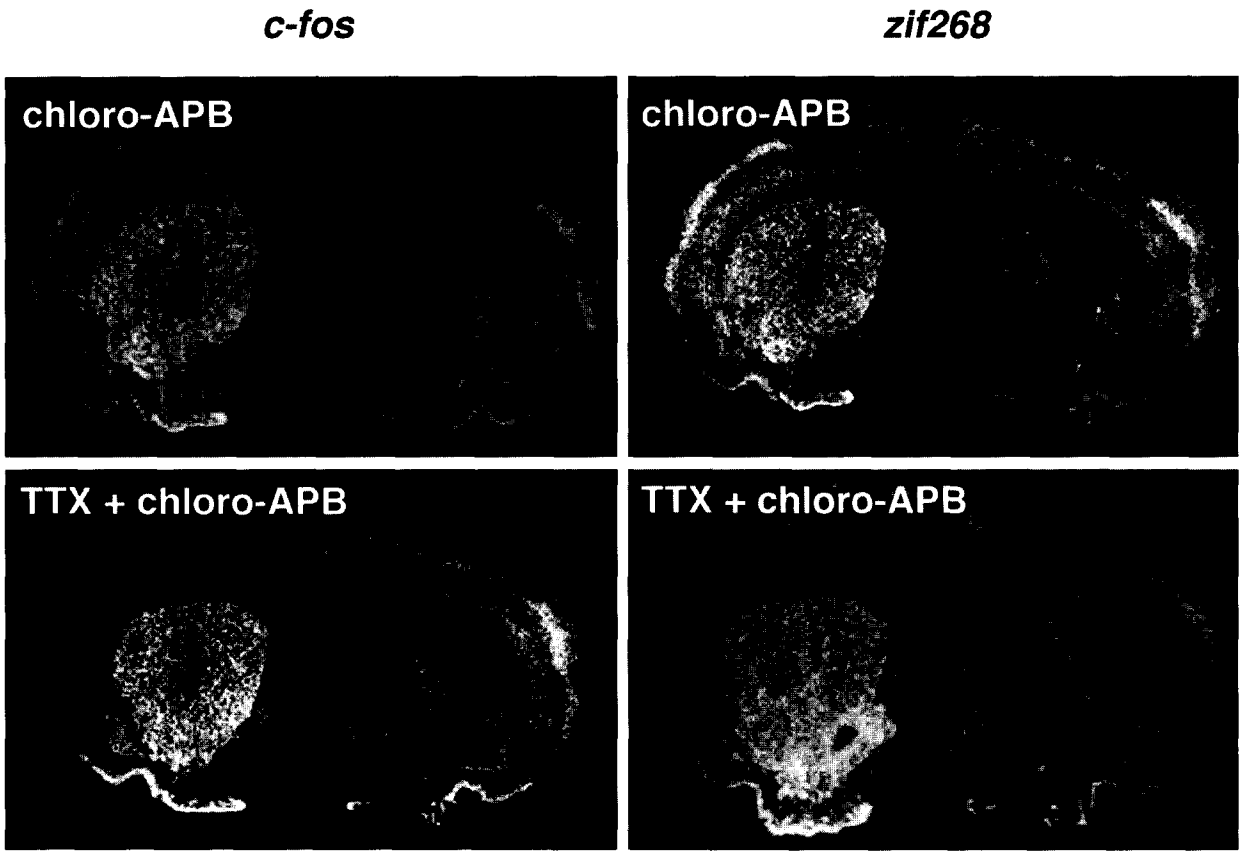

Fig. 4. *In situ* film autoradiogram showing the effects of intrastriatal infusion of TTX (10 μM) via a microdialysis probe implanted in the dopamine-depleted striatum on the expression of c-*fos* and *zif268* induced by systemic administration of the full D_1 dopamine receptor agonist chloro-APB (SKF 82958, 0.5 mg/kg, i.p.). TTX was dissolved in water and then diluted in ACSF and perfused through the microdialysis probe beginning 1 h before the systemic administration of chloro-APB and continuing throughout the 60-min survival period. Control rats received an i.s. perfusion of ACSF and systemic administration of chloro-APB.

of eticlopride (0.5 mg/kg, i.p.) induced the expression of both *zif268* and c-*fos* in the dopamine-depleted striatum (Fig. 6, Table 4). Local infusion of TTX into the dopamine-depleted striatum significantly, but incompletely, attenuated the induction of *zif268* by 59 ± 5.3% (mean ± S.E.M., $n=6$) and c-*fos* by 65 ± 5.7% in rats treated with quinpirole pumps and acute eticlopride (Fig. 6, Table 4; $P<0.05$).

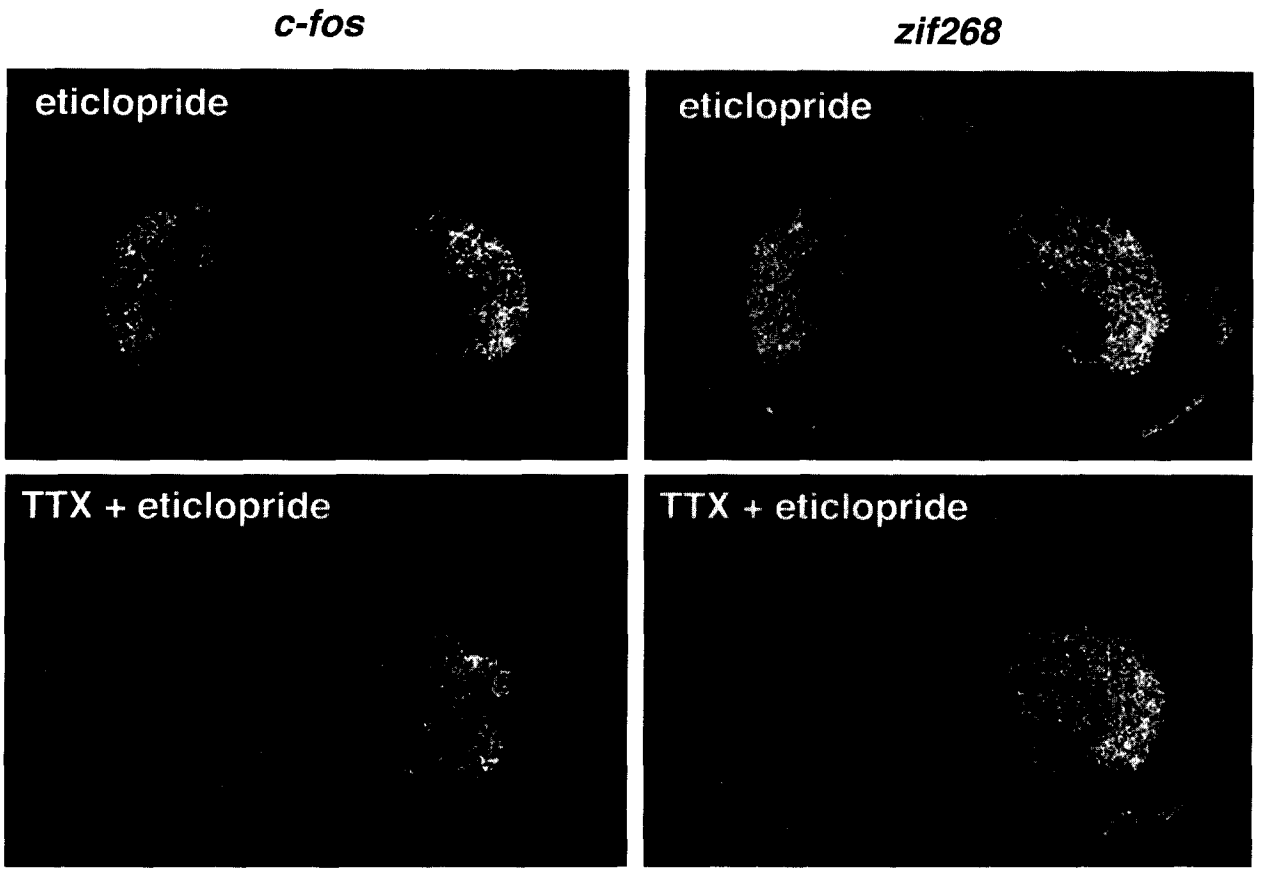

Fig. 5. *In situ* film autoradiogram showing the effects of i.s. infusion of TTX (10 μM) via a microdialysis probe implanted in the right striatum of an intact rat on the expression of c-*fos* and *zif268* induced by systemic administration of the D_2 dopamine receptor antagonist eticlopride (0.5 mg/kg, i.p.). TTX was dissolved in water and then diluted in ACSF and perfused through the microdialysis probe beginning 1 h before the systemic administration of eticlopride and continuing throughout the 60-min survival period. Control rats received an i.s. perfusion of ACSF and systemic administration of eticlopride.

DISCUSSION

Stimulation of D_1 receptors in both the intact and dopamine-depleted striatum results in induction of the immediate early genes c-*fos* and *zif268* in striatonigral neurons.[9,21,51,52] Previous studies have shown that, in intact animals, D_1 receptor-induced immediate early gene expression is sensitive to glutamate receptor blockade.[37,55,58–60] Our previous work, however, indicates that D_1-induced immediate early gene expression in the dopamine-depleted striatum is not blocked by NMDA antagonists.[31] Therefore, the purpose of this study was to determine whether such D_1-induced gene expression in the dopamine-depleted striatum is dependent on input through AMPA/kainate receptors. Local infusion of CNQX into the striatum effectively blocked AMPA/kainate receptors, as evidenced by blockade of AMPA-mediated immediate early gene induction. However, CNQX did not affect D_1-mediated induction of immediate early genes in the dopamine-depleted striatum. To more generally block excitatory input, TTX was locally infused into the striatum. Like CNQX, TTX was ineffective in altering D_1-mediated immediate early gene induction. TTX did, however, block induction of immediate early genes by the D_2 antagonist eticlopride in both the intact and dopamine-depleted striatum. The results suggest that D_1-mediated effects on immediate early gene expression in the dopamine-depleted striatum involve signal transduction mechanisms that operate independent of input through AMPA/kainate receptors.

(±)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptors and D_1 dopamine receptor-mediated gene expression

The finding that local infusion of CNQX did not block D_1-mediated induction of c-*fos* and *zif268* leads to the conclusion that such D_1-mediated gene regulation in the lesioned striatum is not dependent on excitatory input through AMPA/kainate receptors. Such a conclusion is dependent on the validity of the assumption that local infusion of CNQX effectively blocked AMPA/kainate receptors. Although we have no direct measure of AMPA/kainate receptor blockade, the fact that local infusion of CNQX blocks AMPA-induced immediate early gene

Table 3. Effects of intrastriatal infusion of tetrodotoxin on immediate early gene expression induced by systemic administration of D_1 dopamine receptor agonists to dopamine-depleted rats

Treatment	*zif268*	c-*fos*
Dopamine-depleted rats, SKF 38393		
ACSF (i.s.)+SKF 38393 (i.p.)	131.8±4.4 (11)	50.6±2.2
TTX (i.s.)+SKF 38393 (i.p.)	133.2±2.6 (9)	39.1±1.9*
Dopamine-depleted rats, chloro-APB		
ACSF (i.s.)+chloro-APB (i.p.)	86.8±10.1 (6)	75.8±2.4
TTX (i.s.)+chloro-APB (i.p.)	84.0±5.9 (4)	70.4±4.0

TTX or ACSF was infused into the striatum via a microdialysis probe for 2 h, beginning 1 h before the administration of SKF 38393 (2 mg/kg, i.p.) or chloro-APB (0.5 mg/kg, i.p.) to rats with unilateral 6-OHDA-induced depletion of the nigrostriatal dopamine pathway. Rats were killed 1 h after the injection of the D_1 agonist or the vehicle solution and the brains processed for *in situ* hybridization histochemistry. Values are the mean grey values minus white matter labeling (arbitrary units; ±S.E.M.) measured from film autoradiograms of the striatum at the rostrocaudal level containing the track created by the microdialysis probe. Numbers in parentheses are the number of animals per treatment group. *Significantly different from ACSF+SKF 38393, $P<0.05$.

Table 4. Effects of intrastriatal infusion of tetrodotoxin on immediate early gene expression induced by systemic administration of eticlopride to intact and dopamine-depleted rats

Treatment	*zif268*	c-*fos*
Intact rats		
ACSF (i.s.)+vehicle (i.p.)	7.8±4.9 (4)	2.6±0.5
TTX (i.s.)+vehicle (i.p.)	8.3±1.4 (4)	2.9±1.6
ACSF (i.s.)+eticlopride (i.p.)	45.8±2.5 (7)*	16.3±1.8*
TTX (i.s.)+eticlopride (i.p.)	11.7±0.8 (8)	2.2±0.4
Dopamine-depleted rats		
ACSF (i.s.)+vehicle (i.p.)	2.8±1.2 (8)	−0.2±0.6
TTX (i.s.)+vehicle (i.p.)	2.3±0.3 (4)	0.0±1.0
ACSF (i.s.)+quinpirole pump	−3.5±4.1 (6)	−0.2±0.6
ACSF (i.s.)+quinpirole pump+eticlopride (i.p.)	36.1±7.1 (6)*	21.0±3.2*
TTX (i.s.)+quinpirole pump+eticlopride (i.p.)	15.3±3.5 (6)	6.8±1.1

TTX or ACSF was infused into the striatum via a microdialysis probe for 2 h, beginning 1 h before the administration of eticlopride (0.5 mg/kg, i.p.) to intact rats or rats with unilateral 6-OHDA-induced depletion of the nigrostriatal dopamine pathway. Dopamine-depleted rats were given continuous infusions of quinpirole (0.5 mg/kg/day), via an osmotic mini-pump, beginning four to six days before the administration of eticlopride. Rats were killed 1 h after the injection of quinpirole or normal saline vehicle solution, and the brains processed for *in situ* hybridization histochemistry. Values are the mean grey values minus white matter labeling (arbitrary units; ±S.E.M.) measured from film autoradiograms of the infused striatum at the rostrocaudal level containing the track created by the microdialysis probe. Numbers in parentheses are the number of animals per treatment group. *Significantly different from all other groups, $P<0.05$.

expression provides substantive support for the assumption that local infusion of CNQX effectively blocks AMPA/kainate receptors, especially in relation to AMPA/kainate receptor involvement in immediate early gene expression.

The present findings differ somewhat from previous studies that have suggested interactions between AMPA/kainate receptors and D_1 receptor-mediated responses. For example, transection of corticostriatal afferents or systemic administration of the AMPA/kainate receptor antagonist 5,7-dinitroquinoxaline-2,3-dione attenuates amphetamine-induced immediate early gene expression in the striatum.[8,59] Although amphetamine induces immediate early gene expression by a D_1 receptor-mediated process, several differences between those studies and the present study might account for the ability of AMPA antagonists to reduce immediate early gene expression induced by amphetamine, but not a selective D_1 agonist. First, the amphetamine-induced immediate early gene expression is a more subtle response that appears dependent on contributions from multiple circuit components. For example, it has been shown that amphetamine-induced immediate early gene expression is dependent on interactions between D_1 and D_2 dopamine receptor-containing neurons,[54] whereas D_1 dopamine receptor agonist-induced immediate early gene expression in the dopamine-depleted striatum is not.[30] Blockade of AMPA/kainate receptors might disrupt such intercellular interactions, perhaps by blocking the influence of cholinergic interneurons.[15,60] Second, the difference could be a consequence of the fact that the D_1 agonist-induced expression in the dopamine-depleted striatum in the present study is a supersensitive response. At present, the effects of i.s. NMDA and AMPA/kainate receptor blockade on immediate early gene induction in the intact striatum in response to a direct D_1 dopamine receptor agonist have not been determined.

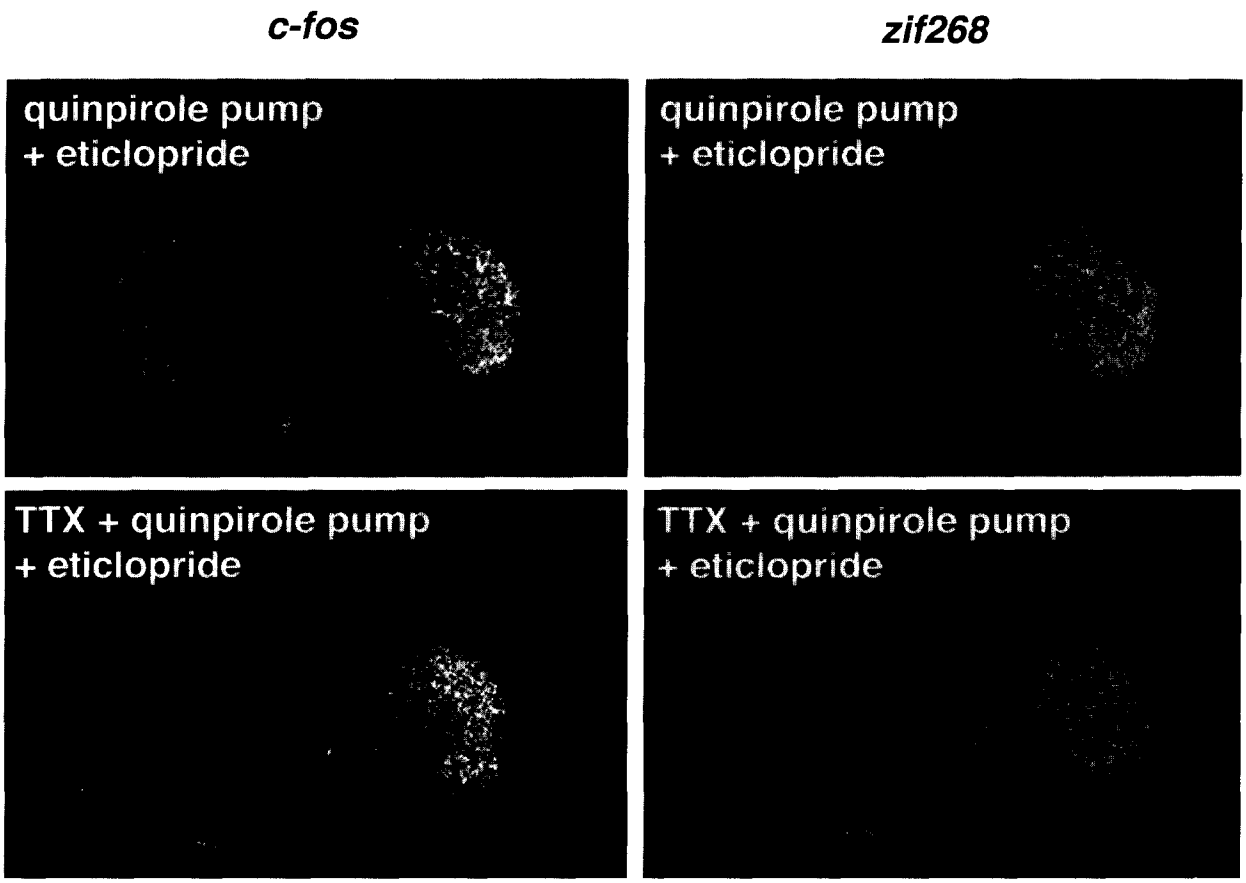

Fig. 6. *In situ* film autoradiogram showing the effects of i.s. infusion of TTX (10 μM) via a microdialysis probe on the expression of c-*fos* and *zif268* in the dopamine-depleted striatum of a rat given a continuous infusion of quinpirole (0.5 mg/kg/day) via an osmotic mini-pump for four to six days and an acute injection of the D_2 dopamine receptor antagonist eticlopride (0.5 mg/kg, i.p.). TTX was dissolved in water and then diluted in ACSF and perfused through the microdialysis probe beginning 1 h before the systemic administration of eticlopride and continuing throughout the 60-min survival period. Control rats received an i.s. perfusion of ACSF, a quinpirole pump and systemic administration of eticlopride.

Such studies will clarify whether there are differences in the role of glutamate receptors between the intact and dopamine-depleted striatum or between the induction of immediate early genes by indirect dopamine receptor agonists versus direct, D_1 dopamine receptor agonists.

Given the evidence suggesting that D_1-induced immediate early gene expression in the dopamine-depleted striatum occurs independent of excitatory afferent input, it seems reasonable to conclude that D_1 dopamine receptor stimulation may directly increase immediate early gene expression in the striatum through the signal transduction pathway involving activation of adenylate cyclase and consequent phosphorylation of the cyclic-AMP response element binding protein.[24,65] Phosphorylated cyclic-AMP response element binding protein has been shown to increase expression of immediate early genes, and is involved in amphetamine-induced, D_1 receptor-dependent induction of immediate early genes in the striatum.[23,36] Although the work of Konradi *et al.*[37] with cultured striatal neurons suggests that this D_1-mediated signal transduction pathway interacts with glutamate input to determine immediate early gene expression, our results indicate that this interaction may not predominate under all conditions.

Effects of tetrodotoxin on dopamine receptor-mediated gene expression

Because D_1 receptor-mediated immediate early gene induction in the dopamine-depleted striatum persisted with blockade of either NMDA[31] or AMPA/kainate receptors, we locally infused TTX in an attempt to more generally block afferent input to, as well as action potential activity in, striatal efferent neurons. Although we have no direct evidence that afferent input was decreased, previous studies using *in vivo* microdialysis have shown that infusion of TTX into the striatum through microdialysis probes of similar design significantly decreases basal

extracellular dopamine,[62] GABA[48] and acetylcholine.[47] Although i.s. infusion of TTX has been reported to have either no effect[61] or to increase[45,46] basal glutamate concentrations in extracellular fluid of the striatum, infusion of 10 μM TTX through a concentric dialysis probe for 40-min blocks evoked glutamate and aspartate release in the striatum,[44] suggesting effective blockade of excitatory afferent input to the striatum. Therefore, local infusion of 10 μM TTX delivered through the dialysis probe provided an additional means of exploring the role of afferent input in D_1-mediated changes in immediate early gene expression in the dopamine-depleted striatum. As was the case with glutamate receptor antagonists, TTX had little effect on D_1-induced immediate early gene expression in the dopamine-depleted striatum, providing further support for the conclusion that this immediate early gene expression is not dependent on afferent input. In addition, to the extent that TTX blocks action potential activity in striatal efferents,[7,12,33] the present findings raise the possibility that D_1 receptor stimulation induces immediate early gene expression independent of changes in action potential activity in striatonigral neurons in the dopamine-depleted striatum. Thus, D_1-induced increases in immediate early gene expression may not always be a valid marker of increased output of the striatonigral pathway.

Because TTX failed to block D_1-induced increases in immediate early gene expression, we chose to also examine whether it could block D_2 dopamine receptor antagonist-induced increases in immediate early gene expression, in an attempt to ascertain whether locally infused TTX could reach effective concentrations when administered in such a manner. TTX infused into the striatum, in fact, blocked the induction of immediate early genes by administration of the D_2 antagonist eticlopride, both in normal animals and in dopamine-depleted rats given a continuous systemic infusion of a D_2 agonist. These findings therefore support our assumption that administration of TTX via the microdialysis probe is capable of blocking immediate early gene expression. In addition, the data suggest that D_2 antagonist-induced immediate early gene expression is dependent on afferents to or action potential activity in striatopallidal neurons. In this case, our findings are consistent with those of others,[6,66] showing blockade of D_2 antagonist-induced immediate early gene expression by glutamate receptor antagonists. Given these positive results, it seems unlikely that the lack of effect of TTX on D_1-induced immediate early gene expression in the present study is an artifact of the experimental approach used.

Afferent activity and striatal immediate early gene expression

The major conclusion of the present study and our previous work[31] is that D_1 dopamine receptor-induced immediate early gene expression in the dopamine-depleted striatum is independent of excitatory afferents. However, several observations from the current experiments indicate that, under certain conditions, immediate early gene expression in the dopamine-depleted striatum is, in fact, dependent on afferent activity. First, glutamate receptor-mediated induction of immediate early genes was demonstrated by infusion of AMPA directly into the striatum. The induction of c-*fos* by AMPA was markedly greater than the induction of *zif268*, providing additional evidence that the expression of these two immediate early genes in striatal neurons may be differentially regulated, as has been suggested by our earlier findings and data from other investigators.[17,31,41,64] The induction of immediate early genes by AMPA was different from the pattern of induction that we observed previously in response to NMDA infusion into the striatum.[31] The local infusion of NMDA resulted in induction throughout the ipsilateral hemisphere rather than in the circumscribed, pericannular region observed with AMPA. Such differences indicate that excitatory input to striatal neurons through various glutamate receptors likely regulates gene expression through different mechanisms, rather than simply through a general increase in cell firing.

Second, induction of immediate early genes by the D_2 antagonist eticlopride appears to be dependent on afferent or action potential activity in the striatum, as it was blocked by local infusion of TTX. This sensitivity to TTX could reflect a dependence of D_2 antagonist-induced immediate early gene expression on cortical or cholinergic activity, as induction of immediate early genes in striatopallidal neurons has been shown to be sensitive to both NMDA receptor blockade[16,66] and muscarinic receptor manipulation.[3,4]

CONCLUSION

A number of different mechanisms are involved in the induction of immediate early genes in the dopamine-depleted striatum. The current findings indicate that excitatory input through AMPA/kainate receptors, activation of D_1 dopamine receptors and blockade of D_2 dopamine receptors are all capable of inducing immediate early genes in the lesioned striatum. However, the present data also demonstrate that whereas the induction of the immediate early genes c-*fos* and *zif268* by D_2 receptor blockade is dependent on afferent input or action potential activity, D_1 dopamine receptor-mediated immediate early gene expression in the dopamine-depleted striatum occurs independent of excitatory afferent input through AMPA/kainate receptors.

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