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Expression of striatal D_1 dopamine receptors coupled to inositol phosphate production and Ca^{2+} mobilization in *Xenopus* oocytes

(rat brain/mRNA/electrophysiology/$^{45}Ca^{2+}$ efflux/phospholipase C)

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ABSTRACT **Expression of central nervous system receptors for dopamine was examined by injection of poly(A)$^+$ RNA (mRNA) from rat striatum into oocytes from *Xenopus laevis*. Electrophysiological measurements in mRNA-injected oocytes indicated that addition of 100 μM dopamine induced an inward current (40–100 nA) that was consistent with the activation of endogenous Ca^{2+}-dependent Cl^- channels. This current was also elicited by addition of the selective D_1 agonist SKF 38393 but not by the selective D_2 agonist quinpirole. Prior addition of the dopaminergic antagonist *cis*-piflutixol completely abolished dopamine-induced currents but had no effect on currents produced by serotonin. Using $^{45}Ca^{2+}$ efflux assays, addition of 100 μM dopamine to injected oocytes stimulated efflux 2- to 3-fold. This increase was mimicked by SKF 38393 and was blocked by the D_1-selective antagonist (+)SCH 23390 but not by the D_2-selective antagonist domperidone. No increase in $^{45}Ca^{2+}$ efflux was seen with 100 μM quinpirole. Size fractionation of striatal mRNA yielded a single peak (2.5–3.0 kilobases) of D_1 receptor-mediated $^{45}Ca^{2+}$ efflux activity in injected oocytes. In addition, dopamine stimulation of oocytes injected with peak fractions and prelabeled with *myo*-[^{3}H]inositol caused a 3-fold increase in [^{3}H]inositol 1,4,5-trisphosphate ([^{3}H]InsP_3) formation. No effect on [^{3}H]InsP_3 production or $^{45}Ca^{2+}$ efflux was observed, however, in injected oocytes incubated with 1 mM N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate. Thus, in addition to D_1 receptors that stimulate adenylyl cyclase, rat striatum contains D_1 receptors that can couple to InsP_3 formation and mobilization of intracellular Ca^{2+}.**

Dopaminergic neurotransmission plays a central role in motor control and a number of behavioral responses (1). Functional abnormalities in dopaminergic neurons in humans have been implicated in various disorders including Parkinson disease, tardive dyskinesia, and schizophrenia. Receptors for dopamine have classically been divided into two subtypes, D_1 and D_2, based on a variety of pharmacological, biochemical, and physiological criteria (1). Dopamine receptors belong to a large "superfamily" of receptors for neurotransmitters and hormones that couple to their signal transduction pathways via guanine nucleotide binding proteins (G proteins) (2). D_1 receptors have been shown to cause activation of adenylyl cyclase and subsequent generation of cAMP (3). Initially, D_2 receptors were described that inhibit the activation of adenylyl cyclase (4). More recent evidence suggests that D_2 receptors are coupled to a number of transduction mechanisms including increased K^+ conductance (5), inhibition of agonist-stimulated inositol phosphate (Ins*P*) formation (6), and inhibition of voltage-dependent Ca^{2+} channels and Ca^{2+} mobilization (6, 7).

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Recently, D_1 receptors have been described in renal tissue that are linked to the activation of phosphatidylinositol-specific phospholipase C (8, 9). Agonist stimulation of D_1 receptors in rat renal tubular membranes has been shown to increase Ins*P* formation independent of the activation of adenylyl cyclase. We have examined the possibility that this transduction pathway also exists for D_1 receptors in the central nervous system (CNS) by examining the functional expression of receptors for dopamine in oocytes from *Xenopus laevis*. *Xenopus* oocytes are particularly suited for the expression of foreign mRNA for receptors that couple to G proteins, which activate phospholipase C (10–12). Oocytes with associated follicle cells contain muscarinic receptors, which couple to G proteins (either G_i or G_o) that are present in mature oocytes (13). Endogenous muscarinic receptors or expressed receptors activate phospholipase C and increase Ins*P*, in particular inositol 1,4,5-trisphosphate (InsP_3), which mediates the release of Ca^{2+} from intracellular stores. Intracellular mobilization of Ca^{2+} in the oocyte leads to the activation of Ca^{2+}-dependent Cl^- channels (12). Thus, expression of receptors may be followed by measurements of Cl^- ion conductance or $^{45}Ca^{2+}$ efflux. In the present study, oocytes were injected with mRNA prepared from rat striatum, a tissue rich in dopaminergic innervation and receptor expression (14). Our results indicate that a D_1 receptor capable of coupling to Ins*P* formation and Ca^{2+} mobilization is present in the CNS and can be expressed from striatal mRNA.

MATERIALS AND METHODS

Preparation of mRNA. Striatal tissue from Sprague–Dawley male rats (175–225 g) was manually dissected, frozen immediately on dry ice, and stored in liquid N_2. Total RNA was isolated from 40–150 striatal pairs, as needed, according to described methods (15). Tissue was rapidly thawed and solubilized in 5.5 M guanidine thiocyanate/25 mM sodium citrate/0.5% sodium lauryl sarcosine/0.2 M 2-mercaptoethanol, pH 7.0 (GNTC solution). DNA was sheared by three passes each through a 19-gauge and a 23-gauge needle. The GNTC lysate was divided (≈20 ml each) and layered directly onto 17 ml of CsTFA solution (cesium trifluoroacetate/0.1 M EDTA, pH 7.0) at a final density of 1.51 g/ml. RNA was separated from DNA and cellular proteins at 25,000 rpm in SW28 rotors for 23–24 hr at 15°C. RNA pellets were dissolved in 0.4 ml of 4 M GNTC solution, and total RNA was precipitated in acetic acid and ethanol. After a second NaCl/ethanol precipitation, poly(A)$^+$ RNA was separated from total RNA by two rounds of oligo(dT)-cellulose chromatography (16).

Abbreviations: CNS, central nervous system; Ins*P*, inositol phosphate(s); Bt_2cAMP, N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate.

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Size fractionation of poly(A)$^+$ RNA was carried out as described (17). Poly(A)$^+$ RNA (100–250 μg) in sterile water was heated at 65°C for 5 min prior to sedimentation through a 10–30% sucrose gradient containing 10 mM Hepes (pH 7.5), 1 mM EDTA, and 0.1% lithium dodecyl sulfate. Samples were centrifuged at 4°C for 18 hr in a Beckman SW41 rotor at 40,000 rpm (200,000 × *g*). Fractions (0.33 ml each) were collected and maintained as 50% ethanol solutions at −80°C. RNA was quantified with a Beckman DU65 UV spectrophotometer (A_{260} unit = 40 μg/ml). Sucrose gradient analysis was performed by measurement of refractive index in identical balance gradients. The size distribution of poly(A)$^+$ RNA in pooled density gradient fractions was determined by denaturing agarose gel electrophoresis (18). Approximately 150 ng of pooled poly(A)$^+$ RNA from three fractions was dried, resuspended in tracking dye containing 50% formamide, and denatured at 95°C for 2 min. Samples were immediately electrophoresed in 1% agarose (SeaKem GTG, FMC) containing 20 mM Mops (pH 6.0), 5 mM sodium acetate, 1 mM EDTA, and 0.66 M formaldehyde. RNA ladder markers (BRL), 0.24–9.5 kilobases (kb), were visualized by staining with ethidium bromide (2 μg/ml). Gradient fractions and markers were transferred electrophoretically onto GeneScreen nitrocellulose paper (NEN) according to the manufacturer's instructions. Sized poly(A)$^+$ RNA fractions were visualized by autoradiography after hybridization with a poly(dT)$_{20}$ oligomer that had been tailed with [^{32}P]TTP (NEN) using terminal deoxynucleotide transferase (Boehringer Mannheim) to a specific activity of ≈5.7 × 10^7 dpm/μg (19).

Oocyte Injection and Culture. Mature oocyte-positive female *X. laevis* were obtained from Nasco (Fort Atkinson, WI) and maintained in aquatic culture. Animals were anesthetized by immersion in ice-cold water for 15–20 min and ovarian lobes were removed by abdominal incision. The largest oocytes were isolated by manual dissection and maintained in modified Barth's solution (MBS) containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 15 mM Hepes, 0.7 mM $CaCl_2$, 0.8 mM $MgSO_4$ and supplemented with 5 mM sodium pyruvate, penicillin (10 μg/ml), streptomycin (10 μg/ml), and fungizone (0.2 μg/ml) (20). After 18–24 hr at 18°C, viable oocytes were injected in the vegetal pole with either 50 nl of sterile water or poly(A)$^+$ RNA prepared from NaCl/ethanol precipitates from individual (1.5 μg) or pooled fractions (3 × 500 ng) and resuspended in sterile water to a final concentration of 1 μg/μl. Oocytes were stored at 18°C in MBS, which was changed daily until the time of assay.

Electrophysiological Assay. Before injection, oocytes were defolliculated by treatment with collagenase (2 mg/ml) in MBS for 1–2 hr at room temperature and were stored in MBS at 18°C thereafter. Oocytes were assayed for changes in membrane current in response to additions of drugs at a voltage-clamped membrane potential of −60 mV. Voltage clamp was maintained by using dual (3 M KCl) electrodes. All drugs, made up fresh in MBS immediately before use, were applied by bath perfusion at the indicated concentrations for 2 min at a flow rate of 1 ml/min. Antagonist perfusions were carried out for 15 min prior to the addition of agonists in the continued presence of the antagonist. Only oocytes with resting membrane potentials of −40 to −60 mV were used.

$^{45}Ca^{2+}$ Efflux Assay. The determination of $^{45}Ca^{2+}$ efflux from injected oocytes was performed with minor modifications as described (10). Oocytes containing follicle cells were incubated in MBS containing 20 μCi of $^{45}Ca^{2+}$ per ml (41 Ci/g; 1 Ci = 37 GBq; NEN) for 18–24 hr. After washing three times with MBS, groups of four or five oocytes were placed in 200 μl of MBS per well of 96-well flat-bottomed plates. With this procedure eight sets of oocytes could be simultaneously processed with an eight-channel adjustable micropipette. Media (180 μl) were collected and replaced every 10 min for 50 min, and then every 5 min thereafter. Drugs were added, as indicated, for 80-, 85-, and 90-min time points. Basal release was defined as the mean release in the 65-, 70-, and 75-min aliquots. Response to 100 μM carbachol at 105 min was measured to assess viability of oocytes that did not respond to other reagents. With this protocol, control $^{45}Ca^{2+}$ efflux ranged from 300 to 650 dpm. Radioactivity in each aliquot was measured in a liquid scintillation counter.

Ins*P* Formation. Ins*P* formation was quantitated essentially as described (11). Oocytes were labeled for 18–24 hr in MBS containing 50 μCi of *myo*-[^{3}H]inositol per ml and then washed three times in MBS. After subsequent incubation in MBS containing 10 mM LiCl for 15 min, 100 μM dopamine was added for an additional 15 min. Incubations were terminated by the addition of perchloric acid to 5% (wt/vol) and disruption of oocytes with a pipette tip while incubating on dry ice. The solution was extracted with trichlorotrifluoroethane/tri-*n*-octylamine (1:1) and Ins*P* were separated by HPLC on a strong anion-exchange column (Beckman) using a linear gradient of 0–0.6 M aqueous ammonium phosphate (pH 3.35) over 30 min. Radioactivity was detected with an on-line liquid scintillation monitor (Radiomatic Instruments and Chemical, Tampa, FL).

RESULTS

Dopamine Stimulation of Membrane Currents in Oocytes. In preliminary experiments, defolliculated oocytes injected with 50 ng of striatal mRNA displayed characteristic neuronal ionic conductances (Na^+, K^+) by 72 hr. At this time, oocytes responded to the addition of 100 μM dopamine with inward currents (40–100 nA) consistent with the opening of endogenous Ca^{2+}-activated Cl^- channels in the oocyte membrane (Fig. 1; data not shown). In addition, oocytes displayed a marked response to the addition of 10 μM serotonin. When

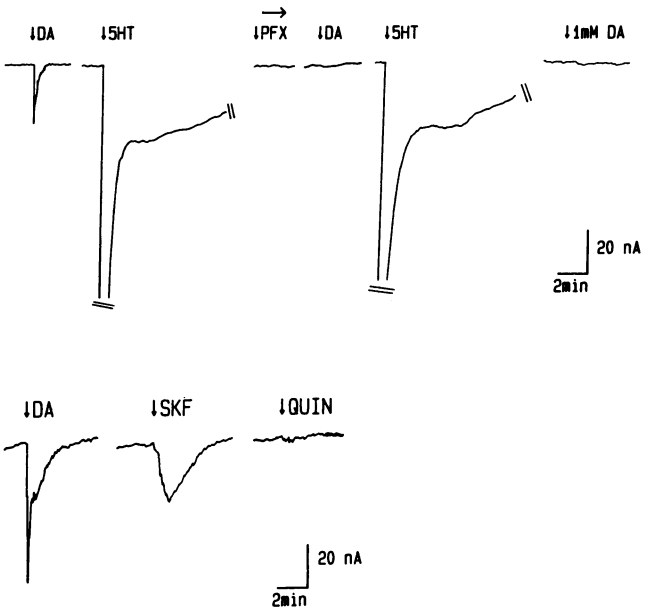

FIG. 1. Electrophysiological responses in oocytes injected with rat striatal mRNA. (*Upper*) Defolliculated oocytes were each injected with 50 ng of striatal mRNA, maintained in MBS, and assayed 72 hr later for changes in membrane currents in response to the application of dopamine (DA) (100 μM alone) or serotonin (5HT) (10 μM alone), and again during *cis*-piflutixol (PFX) (2 μM) perfusion. (*Lower*) Oocytes prepared as described above were assayed electrophysiologically for changes in membrane currents in response to dopamine (DA), the D_1-selective agonist SKF 38393 (SKF), or the D_2-selective agonist quinpirole (QUIN). All concentrations in the bath perfusion were 0.5 mM. Results shown are representative of those observed with two or three oocytes injected with different preparations of striatal mRNA.

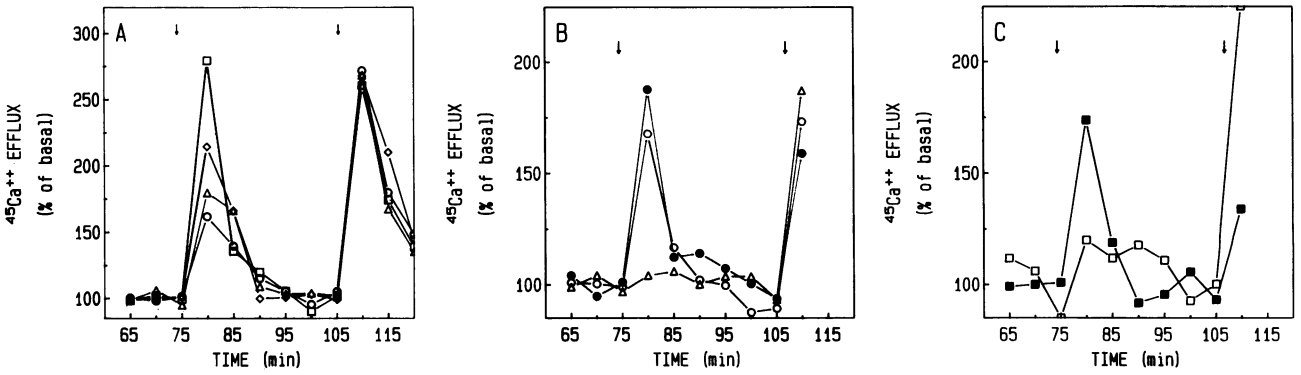

FIG. 2. $^{45}Ca^{2+}$ efflux in oocytes injected with rat striatal mRNA. (*A*) Time course of expression of dopamine-stimulated $^{45}Ca^{2+}$ efflux. Oocytes containing associated follicle cells were prepared and injected as described and then assayed at the times indicated. $^{45}Ca^{2+}$ efflux elicited by 100 μM dopamine (left arrow) was measured 2 (○), 3 (◇), 4 (□), and 5 (△) days after injection. Carbachol (100 μM) was added to each group after 105 min (right arrow) to assess the responsiveness of the oocytes. (*B*) D_1-selective blockade of dopamine-stimulated $^{45}Ca^{2+}$ efflux. Oocytes were prepared and injected as described and then assayed for the ability of subtype-selective antagonists to block $^{45}Ca^{2+}$ efflux elicited by dopamine: 100 μM dopamine alone (●), 100 μM dopamine plus 100 μM domperidone (○), 100 μM dopamine plus 100 μM SCH 23390 (△). Dopamine and receptor antagonists were added after 75 min (left arrow). Carbachol (100 μM) was added after 105 min (right arrow) to assess the responsiveness of the oocytes. (*C*) D_1-selective stimulation of $^{45}Ca^{2+}$ efflux. Oocytes were prepared and injected as described and then assayed for the ability of subtype-selective agonists to stimulate $^{45}Ca^{2+}$ efflux: 100 μM SKF 38393 (■); 100 μM quinpirole (□). Dopamine agonists were added after 75 min (left arrow). Carbachol (100 μM) was added after 105 min (right arrow) to assess the responsiveness of the oocytes. Results shown are representative of those obtained in two experiments.

oocytes were perfused for 15 min with the dopamine antagonist *cis*-piflutixol, subsequent response to the addition of 100 μM to 1 mM dopamine, but not 10 μM serotonin, was abolished (Fig. 1 *Upper*). This failure to respond to subsequent additions of dopamine could not be explained by marked attenuation due to desensitization (data not shown). In a separate experiment, the dopaminergic response could be mimicked by the D_1-selective agonist SKF 38393 but not by the D_2-selective agonist quinpirole (Fig. 1 *Lower*). At no time were responses to dopamine observed in control, water-injected oocytes.

Dopamine Stimulation of $^{45}Ca^{2+}$ Efflux in Oocytes. To further characterize the response to dopamine in oocytes injected with striatal mRNA, experiments designed to measure agonist-stimulated $^{45}Ca^{2+}$ efflux from oocytes were performed. Maximum stimulation of $^{45}Ca^{2+}$ efflux (≈2- to 3-fold) by 100 μM dopamine was observed 4 days after injection (Fig. 2*A*). No response was observed in control, water-injected oocytes (data not shown). Subsequent assays for expression of receptor-mediated activity in oocytes were all performed 4 days postinjection. The dopaminergic response could be blocked by the presence of the D_1 antagonist SCH 23390 but not by the D_2 antagonist domperidone (Fig. 2*B*). A similar stimulation was observed by the addition of 100 μM SKF 38393 but not by the addition of 100 μM quinpirole (Fig. 2*C*). In separate experiments, inclusion of the serotonin receptor antagonists ketanserin or mianserin, or the α-adrenergic antagonist phentolamine at 10 or 100 μM had no effect on $^{45}Ca^{2+}$ efflux stimulated by 100 μM dopamine (data not shown). In addition, no stimulation of $^{45}Ca^{2+}$ efflux was observed in three experiments in which injected oocytes were incubated with 1 mM N^6,$O^{2'}$-dibutyryladenosine 3',5'-cyclic monophosphate (Bt_2cAMP) (data not shown). Thus, these data suggest that rat striatum contains D_1 receptors that stimulate Ca^{2+} mobilization independent of the activation of adenylyl cyclase.

Size Fractionation of Striatal mRNA and Dopamine-Stimulated $^{45}Ca^{2+}$ Efflux in Oocytes. In initial experiments, 100 μg of poly(A)$^+$ RNA was fractionated by size under denaturing conditions over a 10–30% sucrose gradient for 18 hr at 200,000 × *g*. RNA was distributed across the entire gradient with little aggregated material in the last fraction (Fig. 3*A*). RNA recovery was >95%. When equal amounts of RNA (500 ng each) were pooled from groups of three fractions and injected into oocytes, a single peak of dopamine-stimulated $^{45}Ca^{2+}$ efflux activity was observed (Fig. 3*B*). This activity was clearly separated from a single peak of serotonin-stimulated $^{45}Ca^{2+}$ efflux activity present in mRNA prepared from this tissue. Dopamine-stimulated $^{45}Ca^{2+}$ efflux activity was contained in fractions with an average mRNA size of 2.5–3.0 kb, smaller than the size observed for serotonin-stimulated activity, which was 5.0–5.5 kb. In addition, fractions were assayed for stimulation of $^{45}Ca^{2+}$ efflux in the presence of either 10 μM SKF 38393 or 100 μM quinpirole (Fig. 3*C*). The D_1-selective agonist SKF 38393 caused a 3-fold stimulation of $^{45}Ca^{2+}$ efflux activity in identical fractions expressing dopamine-stimulated efflux activity. No effect of quinpirole was observed in any of the fractions.

Dopamine-Stimulated Ins*P* Formation in Oocytes Injected with Sized Striatal mRNA. An additional size fractionation of 250 μg of striatal poly(A)$^+$ was performed under conditions identical to those previously described. Individual fractions were assayed by injection of 50 ng of mRNA into groups of oocytes. A single peak of stimulation of $^{45}Ca^{2+}$ efflux elicited by 100 μM dopamine was observed. As in the previous experiment, the average size of mRNA encoding the expression of D_1 receptors linked to the mobilization of intracellular Ca^{2+} was 2.5–3.0 kb (Fig. 4*A*). When groups of oocytes (20 each) were injected with mRNA from these peak fractions and prelabeled with 20 μCi of *myo*-[^{3}H]inositol per ml for 18–24 hr, subsequent addition of 100 μM dopamine stimulated overall production of [^{3}H]Ins*P* (Fig. 4*B*). In particular, dopamine stimulated a 3-fold increase in the formation of [^{3}H]InsP_3, the second messenger linked to the mobilization of Ca^{2+} from intracellular pools. In three separate experiments, incubation of similarly injected oocytes with 1 mM Bt_2cAMP had no effect on Ins*P* production or the formation of [^{3}H]InsP_3 (data not shown).

DISCUSSION

We have provided evidence that D_1 dopamine receptors in the CNS are capable of coupling to an alternative signal transduction pathway in addition to the stimulation of adenylyl cyclase. mRNA was prepared from rat striatum, a tissue containing very high levels of both D_1 and D_2 receptors (14). Expression of receptors for dopamine was examined by injection of striatal mRNA into oocytes from *X. laevis*, cells capable of efficient expression of foreign receptors coupled to G proteins, which activate phospholipase C (10–12). In

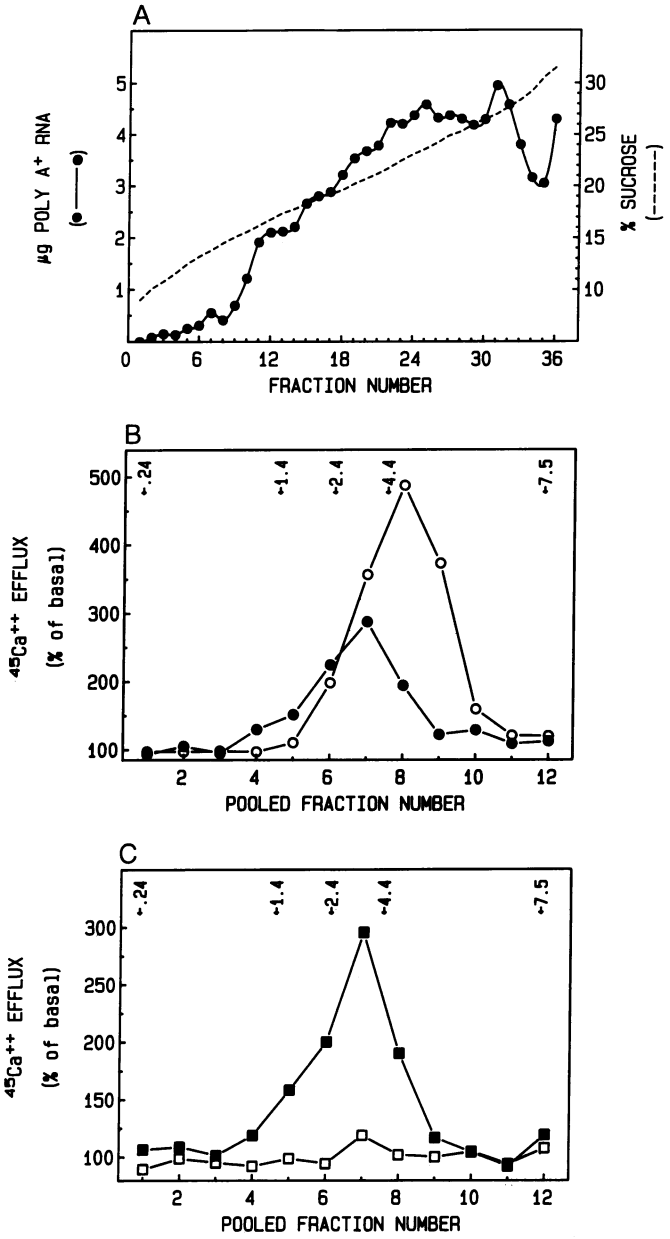

FIG. 3. Dopamine receptor-mediated $^{45}Ca^{2+}$ efflux in oocytes injected with size-fractionated striatal RNA. (*A*) Size fractionation of striatal mRNA. One hundred micrograms of poly(A)$^+$ RNA was separated according to size over a 10–30% sucrose gradient at 200,000 × *g* for 18 hr at 4°C. RNA in each fraction (●) was measured spectrophotometrically (1 A_{260} unit = 40 μg/ml). Measurements of refractive index were obtained from an identical balance gradient to determine percent sucrose (-----). (*B*) Distribution of dopamine- and serotonin-stimulated $^{45}Ca^{2+}$ efflux in oocytes injected with size-fractionated mRNA. Five hundred nanograms from each of three of the above fractions was pooled (starting with fraction 3) and 50 nl of a 1 μg/μl sample was injected into oocytes. $^{45}Ca^{2+}$ efflux in response to 100 μM dopamine (●) or 100 μM serotonin (○) was measured after 4 days in MBS. Positions of RNA size markers (in kb) relative to gradient fractions are indicated above. Size of pooled gradient fractions was determined after agarose gel electrophoresis of fractions and transfer to nitrocellulose. Blots were prehybridized in 0.75 M NaCl/50 mM $NaHPO_4$/5 mM EDTA, pH 7.4 (5× SSPE), containing 50% formamide, 5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone), 0.1% SDS, sheared salmon sperm DNA (250 μg/ml), and yeast tRNA (125 μg/ml) at 45°C for 16–18 hr. Hybridization was continued in the above solution containing 1 × 10^6 dpm of [^{32}P]oligo(dT) probe per ml under identical conditions. Blots were washed three times each for 15 min at 37°C and 45°C in 2X SSPE containing 0.1% SDS. Labeled poly(A)$^+$ RNA was visualized by autoradiography on Kodak X-Omat AR film. (*C*) D_1-selective stimulation of $^{45}Ca^{2+}$ efflux in oocytes injected with size-fractionated striatal mRNA. Oocytes were injected with mRNA from pooled gradient fractions as in *B* and assayed for $^{45}Ca^{2+}$ efflux in response to 100 μM SKF 38393 (■) or 100 μM quinpirole (□). Positions of RNA size markers relative to gradient fractions are indicated above.

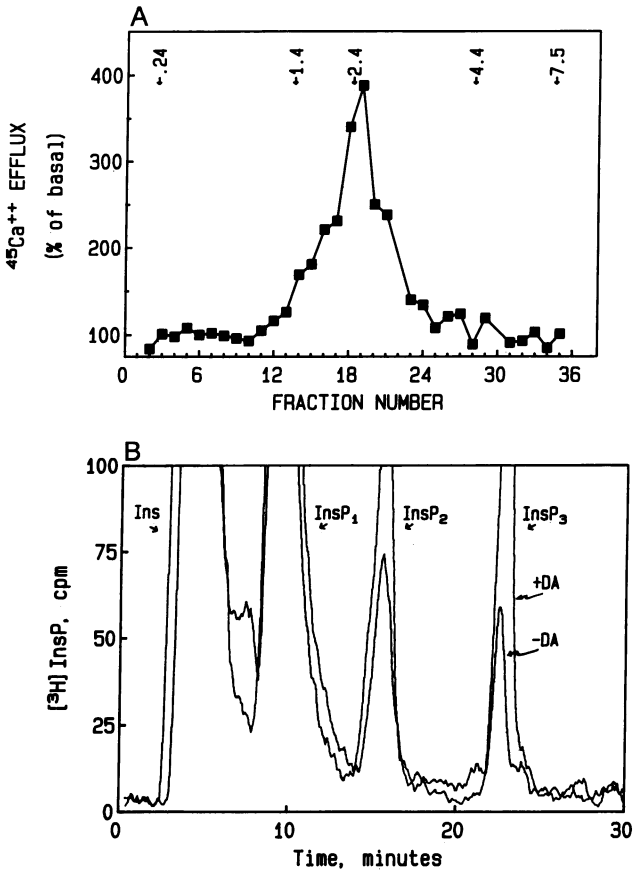

FIG. 4. Dopamine stimulation of Ins*P* formation in oocytes injected with sized-fractionated striatal mRNA. (*A*) Two hundred and fifty micrograms of striatal mRNA was separated by size over a 10–30% sucrose gradient as in Fig. 3. Individual fractions (1.5 μg each) were assayed for the ability to elicit increases in $^{45}Ca^{2+}$ efflux in oocytes in response to 100 μM dopamine. (*B*) Dopamine-stimulated increases in Ins*P* in oocytes injected with size-fractionated striatal mRNA. Groups of 20 oocytes were injected with 50 ng of mRNA from peak fractions (fractions 18 and 19), incubated 3 days in MBS, and then prelabeled with 20 μCi of *myo*-[^{3}H]inositol per ml for 18–24 hr. Oocytes were then incubated in MBS/10 mM LiCl in the absence (−DA) or presence (+DA) of 100 μM dopamine for 15 min at 21°C. Extracted [^{3}H]Ins*P* was separated by strong anion-exchange HPLC and radioactivity was monitored by an on-line liquid scintillation counter. Relative increases in InsP_1, InsP_2, and InsP_3 by dopamine were 1.1-, 1.7-, and 2.8-fold, respectively.

electrophysiological assays, addition of dopamine to injected oocytes produced modest inward currents characteristic of the activation of endogenous Ca^{2+}-activated membrane Cl^- channels. These actions of dopamine were mimicked by a D_1-selective agonist, SKF 38393, and blocked by the nonselective dopamine antagonist, *cis*-piflutixol. *Cis*-piflutixol did not block electrophysiological responses to serotonin, a predominant receptor type expressed in oocytes injected with striatal mRNA. No response was observed to the D_2-selective agonist quinpirole in these experiments.

To further characterize dopaminergic responses in oocytes injected with striatal mRNA, we examined the ability of dopamine to elicit mobilization of intracellular Ca^{2+}. Utilizing $^{45}Ca^{2+}$ efflux assays (17), stimulation of expressed recep-

tors for dopamine caused a 2- to 3-fold increase in $^{45}Ca^{2+}$ efflux from prelabeled oocytes. Pharmacological analysis of dopamine-stimulated efflux revealed that these responses were coupled to the expression of D_1 receptors. Thus, dopamine-stimulated $^{45}Ca^{2+}$ efflux was mimicked by SKF 38393 and blocked by the D_1-selective antagonist SCH 23390. In contrast, $^{45}Ca^{2+}$ efflux stimulated by dopamine was not blocked by the D_2-selective antagonist domperidone, nor was it mimicked by quinpirole. SCH 23390 has been reported to have moderate antagonist activity at serotoninergic and α_1-adrenergic receptors (21), both of which activate phospholipase C in striatum (refs. 22 and 23; data not shown). Serotonin receptor antagonists ketanserin and mianserin, as well as the α-adrenergic antagonist phentolamine, however, had no effect on dopamine-stimulated $^{45}Ca^{2+}$ efflux. Interestingly, no $^{45}Ca^{2+}$ efflux was observed in injected oocytes incubated with Bt_2cAMP, thereby suggesting that dopamine-stimulated efflux was not the result of D_1-mediated activation of adenylyl cyclase.

Size fractionation of striatal mRNA and subsequent expression in oocytes revealed single coincident peaks of dopamine-stimulated $^{45}Ca^{2+}$ efflux activity and increased $InsP_3$ formation. These activities were likewise shown to be coupled to D_1 receptor expression. The size of mRNA encoding these D_1 receptors was 2.5–3.0 kb. This size is very similar to mRNAs, detected in CNS tissues by Northern analysis, encoding the recently cloned rat D_2 receptor (24) and an alternative splice variant of D_2 receptor that we have cloned from a rat striatal cDNA library (25).

The regional distribution of D_1 receptors in the CNS has been widely investigated by ligand binding methods and receptor autoradiographic techniques (14, 26). Interestingly, in some regions there is a poor correlation between the number of D_1 receptors and intrinsic D_1 dopamine-stimulated adenylyl cyclase activity. In particular, the striatum contains the highest number of D_1 receptors in the CNS, yet it demonstrates only a modest stimulation of adenylyl cyclase by dopamine or D_1 agonists—50–100% over basal (27–29). Similarly, the amygdala nucleus contains D_1 receptors, yet it appears to be entirely devoid of dopamine-stimulated adenylyl cyclase (29, 30). In contrast, the retina expresses modest levels of D_1 receptors yet dopamine-stimulated adenylyl cyclase activity is very robust—reaching 200–700% of basal (31, 32). In light of our findings, as well as recent evidence of peripheral D_1 receptors in renal tubular membranes (8, 9) that activate phospholipase C and increase $InsP_3$, it would seem unlikely that the D_1 subtype of dopamine receptors is homogeneous.

In the past few years, cDNAs and/or genes for a number of receptors from the G protein-coupled superfamily have been cloned. In particular, the demonstration of multiple transduction pathways and molecular heterogeneity within the muscarinic (33), serotoninergic (34), and α-adrenergic (35–37) classes of receptors has emphasized the inadequacy of present schemes for classic pharmacological subtyping of receptors. The existence of multiple signal transduction mechanisms for any single (sub)class of receptor suggests that a similar molecular heterogeneity may exist. Future studies, including the cloning of receptor cDNAs and genes will be needed to elucidate the molecular heterogeneity of D_1 receptors coupled to the activation of adenylyl cyclase or phospholipase C. These findings will hopefully provide valuable insight into the role of dopaminergic pathways in a variety of neuropsychiatric disorders.

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1. Creese, I. & Fraser, C. M., eds. (1987) *Receptor Biochemistry and Methodology: Dopamine Receptors* (Liss, New York), Vol. 8. pp. 1–245.
2. Freissmuth, M., Casey, P. J. & Gilman, A. G. (1989) *FASEB J.* **3**, 2125–2131.
3. Kebabian, J. W., Agui, T., van Oene, J. C., Shigematsu, K. & Saavedra, J. M. (1986) *Trends Pharmacol. Sci.* **7**, 96–99.
4. Creese, I., Sibley, D. R., Hamblin, M. W. & Leff, S. E. (1983) *Annu. Rev. Neurosci.* **6**, 43–71.
5. Freedman, J. E. & Weight, F. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3618–3622.
6. Vallar, L. & Meldolesi, J. (1989) *Trends Pharmacol. Sci.* **10**, 74–77.
7. Bigornia, L., Suozzo, M., Ryan, K. A., Napp, D. & Schneider, A. S. (1988) *J. Neurochem.* **51**, 999–1006.
8. Felder, C. C., Jose, P. A. & Axelrod, J. (1989) *J. Pharmacol. Exp. Ther.* **248**, 171–175.
9. Felder, C. C., Blecher, M. & Jose, P. A. (1989) *J. Biol. Chem.* **264**, 8739–8745.
10. Williams, J. A., McChesney, D. J., Calayag, M. C., Lingappa, V. R. & Logsdon, C. D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4939–4943.
11. McIntosh, R. P. & Catt, K. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9045–9048.
12. Snutch, T. P. (1988) *Trends NeuroSci.* **11**, 250–256.
13. Kaneko, S., Kato, K.-I., Yamagishi, S.-I., Sugiyama, H. & Nomura, Y. (1987) *Mol. Brain Res.* **3**, 11–19.
14. Palacios, J. M. & Pazos, A. (1987) in *Receptor Biochemistry and Methodology: Dopamine Receptors*, eds. Creese, I. & Fraser, C. M. (Liss, New York), Vol. 8, pp. 175–197.
15. Okayama, H., Kawaichi, M., Brownstein, M. J., Lee, F., Yokota, T. & Arai, K. (1987) *Methods Enzymol.* **154**, 3–28.
16. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
17. Sumikawa, K., Houghton, M., Smith, J. C., Bell, J., Richards, B. M. & Barnard, E. A. (1982) *Nucleic Acids Res.* **10**, 5809–5822.
18. Davis, L. G., Dibner, M. D. & Battey, J. F., eds. (1986) in *Basic Methods in Molecular Biology* (Elsevier, New York), pp. 143–145.
19. Tu, C.-P. D. & Cohen, S. N. (1980) *Gene* **10**, 177–183.
20. Coleman, A. (1984) in *Transcription and Translation: A Practical Approach*, eds. Hames, D. & Higgins, S. (IRL, Oxford), p. 277.
21. Christensen, A. V., Arnt, J., Hyttel, J., Larsen, J.-J. & Svendsen, O. (1984) *Life Sci.* **34**, 1529–1540.
22. Hoyer, D. (1988) *Trends Pharmacol. Sci.* **9**, 89–94.
23. Jope, R. S. & Li, X. (1989) *Biochem. Pharmacol.* **38**, 589–596.
24. Bunzow, J. R., Van Tol, H. H. M., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K. A. & Civelli, O. (1989) *Nature (London)* **336**, 783–787.
25. Monsma, F. J., Jr., McVittie, L. D., Gerfen, C. R., Mahan, L. C. & Sibley, D. R. (1989) *Nature (London)* **342**, 926–929.
26. Boyson, S. J., McGonigle, P. & Molinoff, P. B. (1986) *J. Neurosci.* **6**, 3177–3188.
27. Battaglia, G., Norman, A. B., Hess, E. J. & Creese, I. (1985) *Neurosci. Lett.* **59**, 177–182.
28. Battaglia, G., Norman, A. B., Hess, E. J. & Creese, I. (1986) *J. Neurochem.* **46**, 1180–1185.
29. Mailman, R. B., Schulz, D. W., Kilts, C. D., Lewis, M. H., Rollema, H. & Wyrick, S. (1986) *Adv. Exp. Med. Biol.* **204**, 53–72.
30. Lackowicz, J. E., Anderson, C. M. & Kilts, C. D. (1989) *Soc. Neurosci. Abstr.* **15**, 1321.
31. De Keyser, J., Dierckx, R., Vanderheyden, P., Ebinger, G. & Vauquelin, G. (1988) *Brain Res.* **443**, 77–84.
32. Watling, K. J. & Dowling, J. E. (1981) *J. Neurochem.* **36**, 559–568.
33. Bonner, T. I. (1989) *Trends NeuroSci.* **12**, 148–151.
34. Hartig, P. R. (1989) *Trends Pharmacol. Sci.* **10**, 64–69.
35. Kobilka, B. K., Matsui, H., Kobilka, T. S., Yeng-Feng, T. L., Francke, U., Caron, M. G., Lefkowitz, R. J. & Regan, J. W. (1987) *Science* **238**, 650–656.
36. Regan, J. W., Kobilka, T. S., Yeng-Feng, T. L., Caron, M. G., Lefkowitz, R. J. & Kobilka, B. K. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6301–6305.
37. Cotecchia, S., Schwinn, D. A., Randall, R. R., Lefkowitz, R. J., Caron, M. G. & Kobilka, B. K. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7159–7163.