Immortalized Murine Striatal Neuronal Cell Lines Expressing Dopamine Receptors and Cholinergic Properties

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Immortalized hybrid cells were generated by somatic cell fusion of 18-d-old embryonic corpus striatum of the mouse strain C57BL/6J with the N18TG2 neuroblastoma. One of the cell populations obtained was treated with a combination of 1 mM n-butyric acid and 10 μM SKF 38393 (a specific D_1 agonist), and a surviving cell population (E1X) was subcloned. Twenty-seven monoclonal cell lines were obtained and screened for the expression of striatal-specific characteristics including γ-aminobutyric acid (GABA), choline acetyltransferase (ChAT), acetylcholine (ACh), mRNA for specific dopamine receptors, and dopamine- and adenosine 3′:5′-monophosphate-regulated phosphoprotein, M_r 32,000 (DARPP-32), and functional D_1 and D_2 dopamine receptors. Neither the parent hybrid cell population (E1X) nor any of the monoclonal cell lines examined expressed GABA levels significantly different than that of the N18TG2 parent neuroblastoma cells (1.36 ± 0.07 μg/mg protein). The range of ChAT activity in the monoclonal hybrid cell lines was 5.5 ± 0.3 to 921.3 ± 97.4 pmol/min/mg protein. Two of the cell lines expressing ChAT activity (X52 and X58) contained ACh (49.64 ± 4.23 and 1.78 ± 0.07 ng/mg protein, respectively). The neuronal origin of four of the monoclonal hybrid lines was shown by their immunoreactivity, following differentiation with 10 μM forskolin, to neurofilament protein, a neuron-specific marker. The monoclonal hybrid cell lines, but not the N18TG2 neuroblastoma, were shown to express an array of D_1, D_2, and D_5 receptor mRNA as well as DARPP-32 mRNA. Two monoclonal cell lines expressed D_1 receptor binding sites (X57, 29.2 ± 4.5 fmol/mg protein and X62, 43.8 ± 6.8 fmol/mg protein) which mediated the stimulation of adenylate cyclase activity. One cell line, X58, expressed only D_2 dopamine receptors (80.9 ± 9.8 fmol/mg protein) which were negatively coupled to adenylate cyclase activity. These findings suggest that the immortalized monoclonal hybrid cell lines are of neuronal origin and have incorporated elements of the medium spiny and cholinergic neurons of the developing striatum.

[Key words: dopamine receptors, neuronal cell lines, ACh, ChAT, corpus striatum, DARPP-32]

The basal ganglia play a central role in the integration of information from the limbic system and neocortex (for reviews see Bannon and Roth, 1983; Graybiel and Ragsdale, 1983; Graybiel, 1990; Gerfen, 1992). The involvement of the basal ganglia in sensorimotor function is evidenced by the clinical sequelae of neurodegenerative diseases including Parkinson's disease and Huntingtons's chorea (Hornykiewicz, 1973, 1979; Seeman et al., 1989). Dysfunction of mesocortical dopaminergic systems has also been implicated in neuropsychiatric disorders including schizophrenia (Seeman, 1987; Carlsson, 1988; Seeman et al., 1993). A number of neuronal compartments within the corpus striatum have been defined on the basis of their morphology, afferent projections, or neurochemical phenotype (Graybiel, 1990; Gerfen, 1992; Stoof et al., 1992). The regulation of the synthesis and release of neurotransmitters within each of these compartments is under complex control from several pathways including the nigrostriatal dopaminergic projection to the medium spiny neurons which comprise the majority of the neuronal population of the corpus striatum. The effects of dopamine within the corpus striatum are mediated by at least two dopamine receptor subtypes, D_1 and D_2. Dopamine receptors have been classified as D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) receptor families based on their molecular sequence and pharmacological properties (for review see Kebabian and Calne, 1979; Andersen et al., 1990; Grandy and Civelli, 1992; Sibley and Monsma, 1992; Gingrich and Caron, 1993).

The study of specific neuronal interactions is limited *in vivo* and in primary cultures of corpus striatum neurons by the heterogeneity of the primary corpus striatum cell population and the limited amounts of tissue available. One approach to this issue has been to employ cell lines which are derived from, or express neurochemical properties of, the brain region of interest. Immortalized cell lines which provide unlimited amounts of tissue have been obtained by spontaneous transformation events or the introduction of transforming oncogenes (Cepko, 1989; Lendahl and Mckay, 1990). Spontaneous transformation has provided a number of widely studied cell lines including the PC12 cell line arising from a rat pheochromocytoma (Greene

and Tischler, 1976). Spontaneous generation of a human neuronal cell line (Ronnett et al., 1990) has also been reported, but such transformations are a rare event. In contrast, retroviral-mediated oncogene transduction may immortalize neuroblasts at a high rate of efficiency, but this approach has not successfully produced cells which express a specific neurotransmitter phenotype (Cepko, 1989; Lendahl and Mckay, 1990; Eves et al., 1992). Neuroblastoma and retinoblastoma cell lines which express dopamine receptors are available (Balmforth et al., 1986, 1988; Monsma et al., 1989; Sidhu and Fishman, 1990; Ivins et al., 1991; Lovenberg et al., 1991) in addition to the COS-1 line which is derived from renal epithelium (Steffey et al., 1991). However, while such cell lines are homogenous and provide an unlimited supply of tissue for biochemical analyses, they are not derived from the brain and therefore may be of somewhat limited utility in the study of neuronal cell–cell interactions in specific brain regions.

Somatic cell fusion is an alternative technique that permits the immortalization of postmitotic neuronal cells from brain regions which express specific neurotransmitter phenotypes (Hammond et al., 1986; Lee et al., 1990a,b; Choi et al., 1991; Crawford et al., 1992). Furthermore, cell lines produced in this manner may be used as models for the examination of the molecular mechanisms governing the differential expression of specific neurochemical phenotypes (Choi et al., 1992). Somatic cell fusion has been used for the generation of adrenergic cell lines from the peripheral nervous system (Greene et al., 1975) as well as those of central nervous system lineage including septal, hippocampal (Hammond et al., 1986, 1990; Lee et al., 1990a,b), and ventral spinal cord (Cashman, 1991) cell lines. In addition, this technique has been used to establish dopaminergic cell lines derived from embryonic murine mesencephalic neurons (Choi et al., 1991) as well as a rat mesencephalic cell line (Crawford et al., 1992). As an approach to developing model systems to provide a stable, homogenous model of primary corpus striatum neurons, we have used the somatic cell fusion technique to generate monoclonal cell lines of neuronal origin from the corpus striatum. The immortalized monoclonal corpus striatum hybrid cell lines described in this report express an array of dopamine receptor mRNA, as well as dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein, M_r 32,000 (DARPP-32) mRNA, functional D_1 and D_2 dopamine receptors, and choline acetyltransferase activity, and synthesize acetylcholine. The neurochemical profiles of the monoclonal hybrid cell lines indicate that the immortalized hybrid cell lines are derived from the fusion of N18TG2 cells with embryonic medium spiny and cholinergic neurons of the developing corpus striatum.

Materials and Methods

Materials

The embryonic mice used in these experiments were of the C57BL/6J strain. Pregnant mice were obtained from controlled matings in a closed colony derived from Jackson Laboratory stock. Mice were housed with a constant light–dark cycle of 12 hr and fed a breeding diet of mouse chow containing 10% fat (Teklab).

All solutions used in tissue culture were filtered through 0.22 μm sterile filters (Nalgene) and stored in sterile bottles at 4°C. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin, and penicillin–streptomycin (5000 units penicillin; 5000 units streptomycin per ml) were obtained from Grand Island Biological Company. DNase was obtained from Worthington Biochemical. Media for cell lines in monolayer culture consisted of DMEM plus 10% (v/v) FBS and 1% (v/v) penicillin–streptomycin unless stated otherwise. Forskolin,

R(+) SKF-38393, Quinpirole HCl, (R+) SCH-23390, and S(−) Eticlopride were obtained from Research Biochemicals International (Natick, MA).

Dissection and somatic cell fusion

Embryonic day 18 C57BL/6J mouse embryos were employed as the source of primary corpus striatum cells. C57BL/6J cells express hypoxanthine phosphoribosyltransferase (HPRT) activity (Greene et al., 1975; Lee et al., 1990a,b). The neuroblastoma fusion partner was the N18TG2 cell line, which is deficient in HPRT and therefore unable to utilize exogenous sources of hypoxanthine for purine synthesis (Greene et al., 1975). Following fusion, the cells were cultured in a medium containing hypoxanthine, aminopterin, and thymidine (HAT medium). Since aminopterin blocks endogenous pathways required for purine and pyrimidine synthesis, the parent N18TG2 cells, which cannot utilize hypoxanthine, do not survive in this medium. Hybrid cells resulting from the fusion of N18TG2 and primary corpus striatum cells have the HPRT defect corrected by the inclusion of chromosomes from parental primary brain cells, thus enabling the hybrid cells to express HPRT and utilize exogenous sources of hypoxanthine. The neuroblastoma cell line was previously obtained through chemical mutagenesis of the N18 neuroblastoma, a subclone of the C1300 neuroblastoma isolated from an A/J mouse (Minna et al., 1975). The N18TG2 cell line expresses negligible levels of choline acetyltransferase (ChAT) activity (Greene et al., 1975; Hammond et al., 1986, 1990; Blusztajn et al., 1992).

Embryos were removed from C57BL/6J mice killed by cervical dislocation on the 18th day of gestation and staged according to the criteria of Gruneberg (1943). The embryonic brain dissection procedure has previously been described in detail (Hemmendinger et al., 1981). The dissociation of primary corpus striatum cells and their fusion with N18TG2 cells was carried out as previously described for the generation of septal cholinergic and mesencephalic dopaminergic cell lines (Hammond et al., 1986; Choi et al., 1991), using a modification of the procedure described by Fournier (1981). Briefly, dissociated CS cells were suspended in 2.5 ml of DMEM containing phytohemagglutinin-P (100 μg/ml) (Sigma, St. Louis). The cell suspension was then pipetted onto a 60 mm culture plate containing N18TG2 cells at approximately 70% confluence. After a 15 min incubation at 37°C, the medium was aspirated and the cells were exposed to 50% (v/v) polyethylene glycol (PEG 1000, Koch-Light) for 1 min at room temperature. These procedures are described in detail in Wainer and Heller (1992). The fusion products were incubated overnight in culture medium (DMEM supplemented with 10% fetal bovine serum) and replated in HAT medium to select for the expression of hybrid cells. Control fusions included N18TG2 × N18TG2 and corpus striatum × corpus striatum cells. Neither of these fusions produced viable colonies. Therefore, any surviving colonies probably arose from hybrid cells derived from the fusion of corpus striatum cells with N18TG2 cells. One of the initial fusion products screened for specific ^{125}I-SCH 23982 binding to D_1 receptor binding sites was treated for 5 d with a combination of 1 mM n-butyric acid and a specific D_1 dopamine receptor agonist (SKF 38393). This treatment was selected in an attempt to increase the expression of D_1 receptors in light of the earlier demonstration of the differentiating effects of n-butyric acid on cultured cells (Prasad and Sinha, 1976) and on somatic cell hybrid cell lines (Choi et al., 1991). In addition, previous studies (Monsma et al., 1990b) have shown that elevation of cyclic adenosine monophosphate (cAMP) using a membrane-permeable cyclic AMP analog leads to an increase in the expression of functional dopamine receptors in a retinoblastoma cell line. The use of a specific D_1 agonist was, therefore, intended to promote the expression of D_1 receptors by stimulation of adenylate cyclase consistent with the report of Monsma et al. (1990b) as well as that of Zhou et al. (1992) of the presence of a cAMP response element in the D_1 receptor gene. While only 5% of the cells survived this treatment, it was possible to obtain a cell population (E1X) from the surviving colonies which does express D_1 and D_2 receptors. This cell population was then expanded and subcloned. Twenty-seven monoclonal cell lines were obtained and selected for further characterization.

Freezing of cell cultures

Cryostorage was performed as described previously for the freezing of mesencephalic hybrid cell lines (Choi et al., 1991). The freezing medium consisted of HEPES-buffered DMEM supplemented with 20% FBS and 10% (v/v) dimethyl sulfoxide (Fisher Scientific, Fair Lawn, NJ).

Radioligand binding studies

Membrane preparation for dopamine receptor characterization. Cells were cultured on 100 mm Falcon plates. Media were aspirated and the monolayer cultures washed three times with harvesting buffer [50 mM Tris–HCl buffer (pH 7.7) at 5°C with 5 mM NaEDTA and 120 mM NaCl]. After washing, the cells were harvested by mechanical disruption with a rubber policeman. The tissue was flash frozen in liquid nitrogen and stored at −70°C. Immediately prior to assay, tissue was thawed slowly on ice, and disrupted by homogenization (Heidolph homogenizer, Polyscience Corp, Niles, IL) with 10 up strokes at low speed in 20 volumes of harvesting buffer. The homogenate was centrifuged at 43,000 × g for 20 min at 4°C (Dupont, Sorvall RC28S with an SM24 head) and the resultant pellet suspended in 50 mM Tris HCl (pH 7.4 at 37°C) with 120 mM NaCl.

Radioligand binding methods for D_1 and D_2 dopamine receptor binding sites were adapted from Sidhu and Kebabian (1985) as described previously (Farfel et al., 1992). D_1 binding sites were labeled with ^3H-SCH 23390 (NEN Dupont, 80 Ci/mmol) or ^{125}I-SCH 23982 (NEN Dupont, 2200 Ci/mmol). D_2 binding sites were labeled with ^3H-spiperone (NEN Dupont, 32.4 Ci/mmol). D_1 receptor binding sites were quantified using saturation studies (six or eight concentrations ranging from 0.02–0.9 nM). Aliquots of tissue (final concentration 100–300 μg protein/ml) were incubated with increasing concentrations of radioligand for 45 min at 37°C in a final volume of 240 μl. Specific binding was defined by 100 μM fluphenazine. Ketanserin (10 μM) was included in all tubes to inhibit radioligand binding to 5-HT_2 serotonin receptor binding sites. For the D_2 assays, increasing concentrations of ^3H-spiperone (six concentrations ranging from 0.04–0.7 nM) were incubated with aliquots of tissue (final concentration 75–250 μg protein/ml) for 45 min at 25°C in Tris–HCl buffer (pH 7.5 at 25°C) with 120 mM NaCl and 0.01% bovine serum albumin. Final assay volume was 300 μl. Specific binding was defined by 10 μM haloperidol. Ketanserin (10 μM) was included in all tubes to inhibit radioligand binding to 5-HT_2 serotonin receptor binding sites.

For all assays, incubation was terminated by filtration under reduced pressure over Whatman GF/B filters (pretreated with 0.3% poly-L-lysine) using a Brandel Cell Harvester modified for radioligand binding assays. Filters were rinsed three times with 5.0 ml ice-cold 50 mM Tris–HCl buffer (pH 7.7 at 25°C). The filters were dried overnight and placed in disposable glass minivials (Research Products International). Three milliliters of a 95% Econofluor/5% Protosol solution (NEN, Waltham, MA) was added and the samples counted by liquid scintillation spectrophotometry (Beckman Model LS 5000TD) with an efficiency of 45%.

Screening of fusion products for dopamine receptor binding sites. Twenty-five cell populations produced by the initial fusion of N18TG2 and primary corpus striatum cells were screened for ^{125}I-SCH 23982 specific binding. Cells were cultured on 100 mm Falcon plates. Harvesting of the cells and membrane preparations for the binding assay were essentially as described by Monsma et al. (1989). The concentration of radioligand used was 0.6 nM.

Subcloning of the fusion products. One of the cell populations derived from the initial fusion of E18 corpus striatum with N18TG2 cells expressed high levels of ^{125}I-SCH 23982 binding sites. This cell population was treated with a combination of 1 mM n-butyric acid and a D_1 agonist (SKF 39893, 10 μM) for 5 d. Less than 5% of the cells survived this treatment. One surviving population (E1X) was selected, expanded, and subcloned for further characterization. Subcloning of single cells from this colony was carried out using a modification of the single-cell plating technique (Puck et al., 1956) as described previously (Choi et al., 1991). Twenty-seven monoclonal cell lines were produced and characterized as described below.

HPLC determination of endogenous γ-aminobutyric acid levels. Measurement of endogenous γ-aminobutyric acid (GABA) levels was performed as described previously (Kontur et al., 1987) using a modification of the method of Lasley et al. (1984). Briefly, cells were removed from the culture plates by mechanical disruption in 1 ml of 0.008 mg/ml α-aminobutyric acid. The cell suspension was then sonicated and immediately stored at 4°C. An aliquot of the supernatant was derivatized with o-phthaldialdehyde and the indole derivative was subsequently separated and quantified by high-performance liquid chromatographic analysis with electrochemical detection (HPLC-ED). The chromatographic system consisted of a Milton Roy minipump (model 396), a 5 μm octadecyl 4.6 × 50 mm reverse-phase column (IBM), and an amperometric detector (BAS LC4) with a glassy carbon electrode (BAS

M-800). The mobile phase consisted of a sodium monobasic phosphate buffer (0.1 M) containing EDTA (0.134 M) and 35% (v/v) HPLC grade methanol at a pH of 5.6. The carbon electrode was maintained at a potential of +0.8 V versus the silver chloride reference electrode with a sensitivity of 20 nA/V.

Choline acetyltransferase activity and endogenous acetylcholine content. Choline acetyltransferase (ChAT) activity was measured using a modification of the method of Fonnum (1975) (Hammond et al., 1986, 1990). ChAT specific activity was expressed as picomoles of acetylcholine formed per minute per milligram protein. Background signal was determined by assaying citrate-phosphate buffer instead of cell extract. Acetylcholine (ACh) levels were determined by HPLC-ED with an enzymatic reactor containing acetylcholinesterase and choline oxidase based on the method of Potter et al. (1983). The materials used were obtained from Bioanalytical Systems Inc. (West Lafayette, IN).

Identification of dopamine receptor and DARPP-32 mRNA expression by polymerase chain reaction. Oligonucleotide primers were synthesized on an Applied Biosystems DNA synthesizer. Given the high degree of identity across species (for example, the mouse D_2 sequence shows 97% nucleic acid homology with the rat D_2 sequence; Mack et al., 1991), dopamine receptor-specific oligonucleotides used for this study were primarily derived from rat receptor sequences unless the mouse sequence was known. All of the chosen probes generated the predicted size fragment which was further verified using internal oligonucleotides as hybridization probes. For the D_1 receptor, sequences were derived from Monsma et al. (1990a) and included: o654, 5′-TCACTGCT-CATCCTGTCCAC, identical to nucleotides 503–522; and o643, 5′-GAGCACATGATGTCAAAGGC, which is complementary to nucleotides 713–732. The D_2 receptor primers o197, o198 were as described by O'Malley et al. (1990). The D_3 receptor primers were derived from Sokoloff et al. (1990) and included: o580, 5′-TGGGCTATGGCAT-CTCTGAGTCAGCT, identical to nucleotides 76–101 and o198 noted above, which is also complementary to the rat D_3 nucleotides 400–423. The D_4 primers (o415, o416, o474) were as described (O'Malley et al., 1992). The D_5 (D_{1B}) primers were derived from Tiberi et al. (1991) and included o644, 5′-ACTGGGACCCGCGCAGGT, identical to nucleotides 99–119 and o643, the sequences of which are conserved between the D_1 and D_5 receptors (nucleotides 346–365 of the rat D_5 receptor; Tiberi et al., 1991). The DARPP-32 primers were 5′-CTGTGCCTA-TACGCCCCCATC and 5′-GGGATGCTGAGGTTCCTCTCCAGGC-TCAC

RNA preparation and standardization were exactly as described by Mack et al. (1991). PCR amplification and analysis were as described by O'Malley et al. (1990, 1992) for the D_2 and D_4 receptors, respectively. Temperatures for the D_3 PCR protocols were 93°C for 90s, 61°C for 60s, and 72°C for 90s. The D_1 and D_5 parameters were 93°C for 60s, 54°C for 60s, and 72°C for 60s. The DARPP-32 parameters were 94°C for 60s, 60°C for 60s, and 72°C for 90s. Oligonucleotides were endlabeled and then added to the PCR mixtures. Each template and primer set was optimized to ensure linearity of exposure. PCR products were separated on a 5% polyacrylamide gel which was subsequently dried and exposed to x-ray film. Three cell lines (X57, X58, and X62) were selected for further characterization based on their expression of dopamine receptor mRNA.

Adenylate cyclase assay. Assays of receptor-mediated adenylate cyclase activity in corpus striatum hybrid cell membranes were modified from the original methodology (Childers, 1985) using ^3H-ATP as a substrate. The ^3H-cyclic AMP reaction product was quantitated by HPLC as described by Childers (1985). Previously frozen (−70°C) tissue was homogenized in 50 volumes of ice-cold 50 mM Tris buffer (pH 7.7 at 4°C) with 2 mM EGTA and 5 mM $MgCl_2$, with a glass-teflon homogenizer. Homogenates were centrifuged at 43,000 × g for 20 min. The resultant pellets were resuspended in adenylate cyclase buffer (50 mM Tris–HCl with 5.0 mM $MgCl_2$, and 2 mM EGTA, pH 7.4) and kept on ice prior to use for adenylate cyclase assays. The membrane preparations were incubated with reaction mixture (160 μl) which contained 30 μM cyclic AMP, 10 μM ATP, 3 μM isobutylmethylxanthine, 5 mM creatine phosphate, 25 U/ml of creatine phosphokinase, 20 μM GTP, 0.04% bovine serum albumin, and 1 μCi ^3H-ATP together with 25–50 μg membrane protein and various drug additions to a total volume of 240 μl. The reaction was initiated by the addition of the ^3H-ATP, incubated at 30°C for 10 min, and terminated by placing the tubes in a boiling water bath for 2 min. The tubes were cooled on ice for 5 min and 0.75 units of adenosine deaminase was added. After a 5 min incubation at 30°C, the tubes were transferred onto ice and the excess remaining ^3H-

ATP was removed by the addition of $Ba(OH)_2$ and $ZnSO_4$, with 5 min between each addition. Blank levels were determined by boiling the tissue for 5 min before continuing with the assay. The tubes were centrifuged at 10,000 × g for 15 min, and the supernatants transferred into a parallel set of tubes for automatic sample injection into the HPLC column. Each tube was run in triplicate and the means of the control and experimental tubes compared by a paired sample t test.

HPLC apparatus (Beckman model 110B pump) was connected to a Rainin Microsorb 3 μm C-18 reverse-phase column together with a C-18 guard column. Mobile phase consisted of 0.8 M sodium acetate, pH 5.0, with 12% methanol at a flow rate of 1 ml/min. Samples were injected (220 μl volume) by a Spark Holland Marathon Autosampler which maintained sample temperature at 5–10°C. The cyclic AMP peak eluted 4.1 min after injection and was collected by a Varex model SF2120 fraction collector. Unlabeled cyclic AMP was detected at 254 nm through an 8 μl flow cell (Beckman Model 153 UV Detector) with a sensitivity of 0.16 AU full scale. ^{3}H-cyclic AMP, collected in three fractions of 0.25 ml, was transferred to a glass scintillation vial to which 8 ml of CytoScint scintillation fluid (ICN Biomedicals) was then added. Radioactive decay was measured on a Beckman LS liquid scintillation counter at an efficiency of 45%.

Under these conditions, more than 90% of the cyclic AMP was recovered as determined from studies using added ^{3}H-cyclic AMP.

Aggregation. The ability of the hybrid cells to aggregate in rotation-mediated cell culture was tested as described by Choi et al. (1991), adapted from the procedure described by Moscona (1961) for examining cell–cell interactions. After 36 hr in culture, the aggregated cells were transferred to a depression slide and photographed.

Differentiation and immunocytochemistry. The clonal hybrid cell lines X57, X58, and X62 and the parent neuroblastoma line N18TG2 were plated at a density of 5–20 × 10⁴ cells per 60 mm culture dish. The cells were maintained in culture for up to 5 d in the presence of 1 mM n-butyric acid, which causes some cultured cells to differentiate (Prasad and Sinha, 1976; Choi et al., 1991), or 10 μM forskolin, which maximally stimulates adenylate cyclase activity, or vehicle (0.1% dimethylsulfoxide). Cultures were examined for the expression of the neuronal marker neurofilament protein (NFP) and a glial marker, glial fibrillary acidic protein (GFAP). Immunostaining was performed according to the peroxidase–antiperoxidase method of Sternberger (1979) as described previously (Choi et al., 1991). The antibodies used were the monoclonal antibody 4.3F₀, which reacts with the carboxyl-terminal domains of the high-molecular-weight neurofilament subunits NF150 and NF200 NFP, as well as 2.2B₁₀.₆ directed against GFAP (Trojanowski et al., 1983).

Protein determination. Proteins were determined using the commercially available Pierce assay (Pierce, Rockford, IL) with bovine serum albumin as a standard.

Data analysis. Saturation isotherms were fitted by a single-site model. Radioligand affinity (K_d) and the density of binding sites (B_{max}) were obtained using weighted Eadie–Hofstee plots as described by Zivin and Waud (1982). Computer-assisted analysis of ^{3}H-SCH 23390 and ^{3}H-spiperone binding isotherms indicated a single, saturable site of interaction. Specific binding ranged from 50 to 80%, varying as a function of the concentration of radioligand and density of binding sites.

Results

Twenty-seven immortalized monoclonal corpus striatum hybrid cell lines were generated from the cell population E1X. The properties of four of the most extensively characterized monoclonal hybrid cell lines (X52, X57, X58, and X62) are summarized in Table 1.

Neurochemical characterization of immortalized monoclonal corpus striatum hybrid cell lines

GABA levels were measured in three monoclonal cell lines (X57, X58, and X62). None of these cell lines demonstrated GABA levels significantly different from that detected in either the N18TG2 parent cell line (1.36 ± 0.07 μg/mg protein, n = 4) or the cell population (E1X) from which the hybrid cells were derived (1.22 ± 0.16 μg/mg protein, n = 5).

The cell lines subcloned from the E1X cell population were also screened for the expression of the cholinergic marker ChAT.

Table 1. Characterization of monoclonal hybrid cell lines derived from somatic cell fusion of embryonic corpus striatum with N18TG2 neuroblastoma cells

Property	Cell line				
	N18TG2	X52	X57	X58	X62
Neurite formation	–		+	+	+
Aggregate formation	–	+	+	+	+
Immunocytochemistry					
NFP	–	+	+	+	+
GFAP	–	–	–	–	–
Neurochemistry					
ChAT activity	–	+	+	+	+
ACh	–	+	–	+	–
GABA			#	#	#
Dopamine receptor mRNA					
D1	–	+	+	–	+
D2	–	+	+	+	–
D3	–	–	–	–	–
D4[a]	+	–	+	+	+
D5	–	–	+	–	+
Functional dopamine receptors					
D1	–		+	–	+
D2	–		–	+	–
DARPP-32 mRNA	+[a]	+	+	+	+

Summary of the properties of selected corpus striatum hybrid lines. Symbols: +, presence of trait; –, absence of trait; spaces, line has not been examined for trait; #, not significantly different from N18TG2 parent cells.

[a] Trace only; detected after 4 d exposure of autoradiograms.

The results of these experiments are shown in Figure 1. The original cell population (E1X) from which these cell lines were subcloned expressed higher levels of ChAT activity (161.29 ± 14.6 pmol/min/mg protein) than some of the subclones examined. Most of the monoclonal hybrid cell lines examined expressed levels of ChAT activity comparable to that observed in the parent N18TG2 cells (see Fig. 1). In contrast, a number of monoclonal cell lines (X52, X58) expressed substantial levels of enzyme activity ranging from 5.5 ± 0.3 to 921.3 ± 97.4 pmol/min/mg protein (X52).

Endogenous ACh levels were measured (Table 2) in two monoclonal cell lines which expressed high levels of ChAT activity (X52 and X58). Both the X52 (49.64 ± 4.23 ng/mg protein) and X58 (1.78 ± 0.07 ng/mg protein) cells contained detectable endogenous levels of ACh. Under these conditions no ACh was detected in the X57 or X62 cell lines, although each of these cell lines demonstrated ChAT activity higher than that observed in the N18TG2 cells. No ACh was detected in the N18TG2 neuroblastoma. The 20-fold greater levels of ACh in X52 and X58 were not correlated with the absolute difference in ChAT activity in the two cell lines.

Dopamine receptor and DARPP-32 mRNA expression in immortalized monoclonal corpus striatum hybrid cell lines

The expression of D_1, D_2, D_3, D_4, and D_5 dopamine receptor mRNA and DARPP-32 mRNA was analyzed using reverse transcription (RT) PCR. For each transcript, standardized RNAs prepared from the hybrid cells were processed simultaneously to allow for direct comparison of band intensities. An arbitrary scale of 1+ to 4+ was chosen to represent the relative intensity
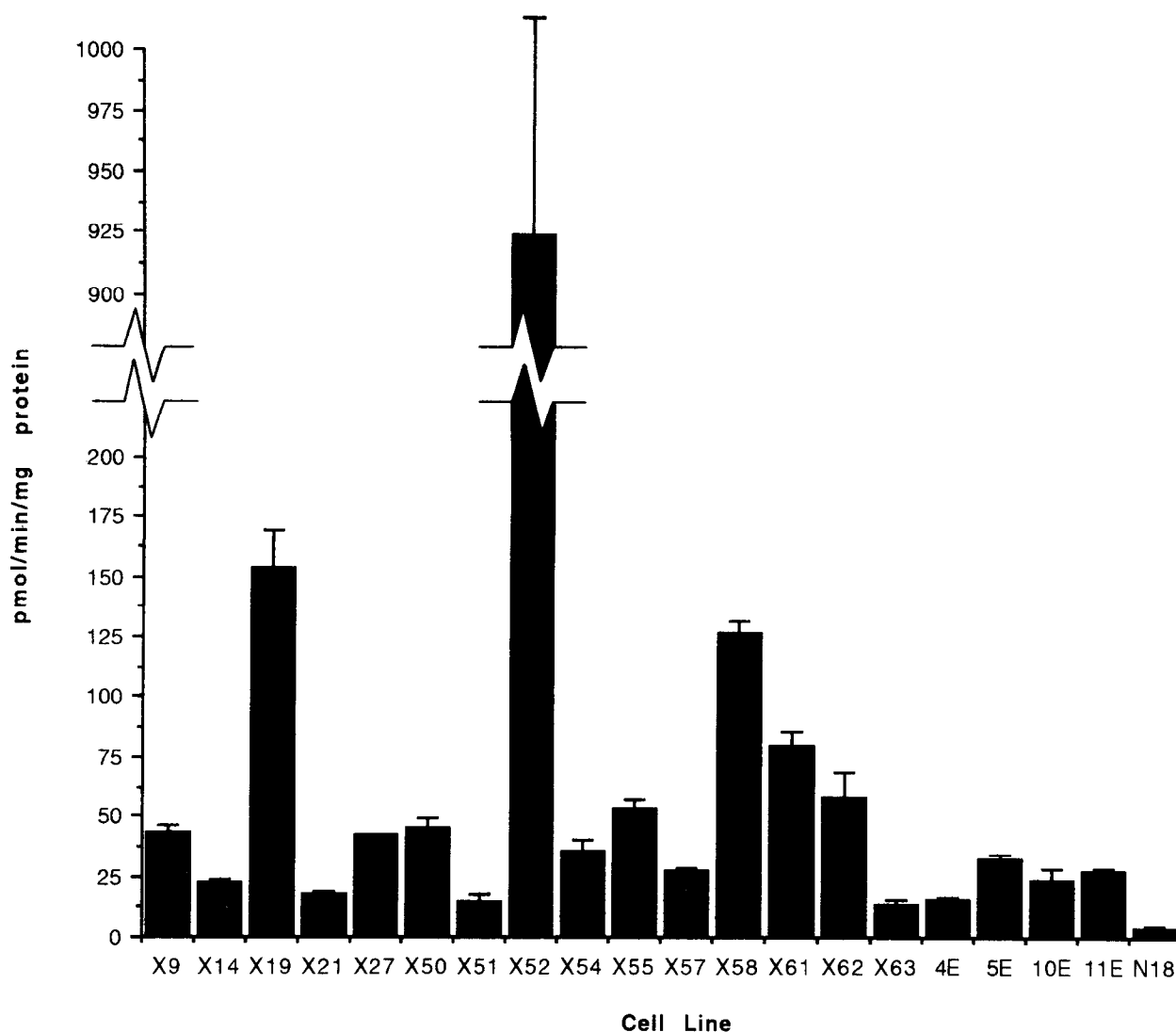
Figure 1. Choline acetyltransferase (ChAT) activities (picomoles of ACh formed per minute per milligram of protein) of E18 corpus striatum hybrid cell lines and the parent N18TG2 neuroblastoma cells. Corpus striatum hybrid cell lines expressing less than 15 pmol/min/mg protein of ChAT activity are not represented in this figure. The values shown are means ± SEM (n = 3).

of individual bands. The results from this analysis for each receptor subtype and DARPP-32 are presented in Table 3. Representative autoradiograms for the D_1 receptor are shown in Figure 2. As indicated, no D_3 mRNA was detected in any of the monoclonal cell lines. Low levels of D_4 mRNA were detected in all the cell lines except X52, albeit with very long exposure times (4 d). Although D_4 receptor mRNA was present, this amount of message is apparently insufficient for detectable protein expression by radioligand binding assays since subsequent experiments could detect no specific binding. Most of the cell lines express a combination of receptor mRNA transcripts. However, several monoclonal lines appear to express only one receptor subtype other than D_4. For example, the cell lines 4E, 5E, 9E, 10E, 11E, X1, X14, and X15 express only the D_1 transcripts. The X21 and X58 cells express only D_2 mRNA, and one cell line (3E) appears devoid of all dopamine receptors except for trace levels of the D_4 transcript. Negligible levels of DARPP-32 mRNA (Table 3) were detected in the parent N18TG2 cells and the cell population (E1X) from which the

monoclonal lines were generated. Some of the monoclonal cell lines (3E, X1, X4, X9, X17, X21) expressed a trace or no transcript. Other monoclonal hybrid cell lines (5E, 9E, 10E, 11E) expressed a robust signal. D_2 dopamine receptor mRNA has also been detected by Northern blot analysis in some of the monoclonal hybrid cell lines (J. S. Fink, personal communication). In addition, in the corpus striatum hybrid cell line X57, by RT-PCR techniques using a range of primer sets which amplify both the 5' untranslated region and other regions of this transcript it has been possible to demonstrate the presence of bands consistent in size with D_1 receptor mRNA, indicating that this cell line does contain a full-length mRNA transcript encoding the D_1 receptor.

D_1 and D_2 dopamine receptor binding site expression in immortalized monoclonal corpus striatum hybrid cell lines

Based on the high levels of ChAT activity and expression of dopamine receptor mRNA observed in subclones X57, X58, and X62, these monoclonal cell lines were then examined for

Table 2. Endogenous acetylcholine (ACh) levels in N18TG2 cells and corpus striatum hybrid cell lines

Cell line	Endogenous ACh levels (ng/mg protein)
X52	49.64 ± 4.23
X58	1.78 ± 0.07
X57	Not detected
X62	Not detected
N18TG2	Not detected

ACh levels in cell homogenates were quantitated by high-pressure liquid chromatography with electrochemical detection. Values represent the mean ± SEM (X52, $n = 5$; X58, $n = 3$).

the expression of D_1 and D_2 dopamine receptor binding sites. D_1 binding sites were observed in the X57 (29.2 ± 4.5 fmol/mg protein) and X62 (43.8 ± 6.8) cells, but no D_2 binding sites were detected. The affinity (K_d) of the radioligand for these D_1 sites was 0.24 ± 0.02 nM (X57) and 0.23 ± 0.06 nM (X62). In the case of the cell line X58, which expressed only the D_2 transcript (Table 3), D_2 binding sites were observed (80.9 ± 9.8 fmol/mg protein). The affinity of the radioligand for the D_2 binding sites was 0.08 ± 0.01 nM.

D_1 and D_2 dopamine receptor–linked adenylate cyclase activity in immortalized monoclonal corpus striatum hybrid cell lines

Given the presence of D_1 and D_2 dopamine receptor binding sites in the hybrid cell lines, studies were conducted to determine whether these receptors were functional by examining their ability to modulate adenylate cyclase (AC) activity (Table 4). D_1 receptor-mediated modulation of AC activity was observed in both the X57 and X62 lines in the presence of a specific D_1 agonist [R(+)SKF 38393, 10 μM]. This stimulation was blocked by the addition of a specific D_1 antagonist (SCH 23390). D_2 receptor-mediated attenuation of forskolin-induced AC activity was detected in the X58 line and was abolished by preincubation with a D_2 receptor antagonist, eticlopride, suggesting that the effect is mediated by D_2 receptor. No receptor-linked stimulation of adenylate cyclase activity was observed in the N18TG2 cells under these conditions.

Cell morphology, growth characteristics, and expression of cytoskeletal markers in immortalized monoclonal corpus striatum hybrid cell lines

In monolayer cultures, lines X57 and X58 grew as clusters of cells connected by a network of processes (Fig. 3A,B). The hybrid X62 cell line grew as a flat monolayer of cells (Fig. 3C). The neuroblastoma cells had flat somata, short processes, and were dispersed as a monolayer (Fig. 3F). The population-doubling time of the hybrid cells and the N18TG2 cells was approximately 24 hr. The neuronal character of the hybrid cell morphology was examined by screening these cells for neurofilament expression, a specific marker for neurons (Lee et al., 1982). Following treatment for 48 hr with forskolin (Fig. 3D) or for 1 week with butyric acid (Fig. 3E), the hybrid lines, but not the N18TG2 cells, expressed prominent, neurite-like processes which reacted with monoclonal antibody 4.3F₉ to neurofilament units NF150 and NF200. Photomicrographs (Fig. 3D,E) are shown for differentiated X57 cells only, although a similar response was observed in both the X58 and X62 cells. Neither the hybrid nor the neuroblastoma cells in the presence of either forskolin

Table 3. Dopamine receptor subtype and DARPP-32 mRNA expression in monoclonal hybrid corpus striatum cell lines

Cell line	mRNA					
	D_1	D_2	D_3	D_4[a]	D_5	DARPP-32
N18TG2	−	−	−	+	−	tr
X1	+	−	−	+	−	tr
X4	+	+	−	+	−	−
X9	+	+	−	+	−	tr
X13	+	+	−	+	−	++
X14	+	−	−	+	−	+
X15	+	−	−	+	−	+
X17	+	+	−	+	−	tr
X18	+	+	−	+	−	+
X19	++	+	−	+	−	++
X21	−	+	−	+	−	−
X24	++	+	−	+	−	+
X27	++	+	−	+	+	++
X50	++	+	−	+	+	+
X51	+	+	−	+	+	++
X52	++	+	−	−	−	+
X54	++	+	−	+	+	++
X55	+	+	−	+	−	+
X57	++++	+	−	+	−	++
X58	−	++	−	+	−	++
X61	++	−	−	+	+	++
X62	+	−	−	+	+	++
X63	−	+	−	+	+	+
3E	−	−	−	+	−	tr
4E	++	−	−	+	−	++
5E	+	−	−	+	−	+++
9E	+	−	−	+	−	+++
10E	+	−	−	+	−	+++
11E	+	−	−	+	−	+++

The expression of D_1, D_2, D_3, D_4, and D_5 dopamine receptor and dopamine- and adenosine 3′ : 5′-monophosphate-regulated phosphoprotein, M_r 32,000 (DARPP-32), mRNA was examined in monoclonal striatal hybrid cell lines using reverse transcription polymerase chain reaction techniques. An arbitrary scale of + to ++++ was chosen to represent the relative intensity of individual bands.

[a] Detected only after 4 d of exposure. tr, trace.

or butyric acid stained with the monoclonal antibody 2.2B₁₀.₆ to glial fibrillary acidic protein.

The monoclonal hybrid cell lines were examined for their ability to form aggregates in a three-dimensional rotation-mediated culture system. In the cell reaggregation system as described by Moscona (1961), N18TG2 cells (Fig. 4D) showed minimal aggregation. However, each of the corpus striatum hybrid cell lines examined (X52, X57, X58, and X62) exhibited reasonably well formed aggregates (Fig. 4A–C), whose mor-
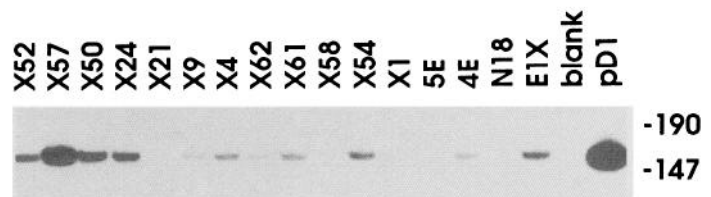

Figure 2. Representative autoradiograms of the reverse transcription–polymerase chain reaction (RT-PCR) D_1 dopamine receptor products from monoclonal hybrid corpus striatum cell lines.
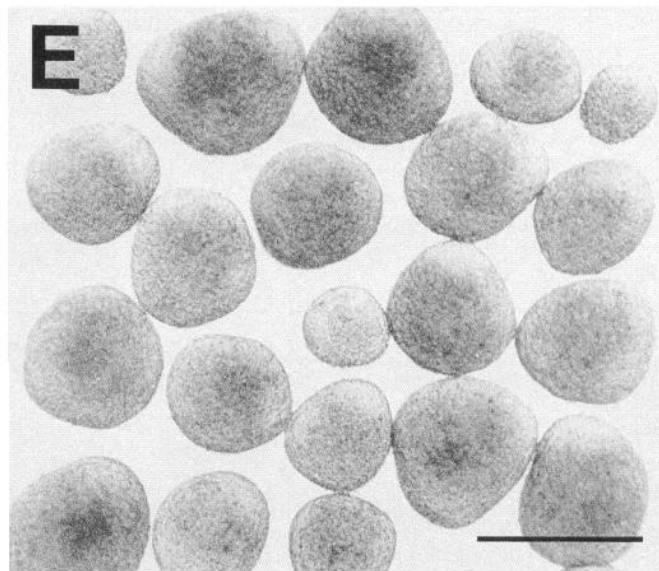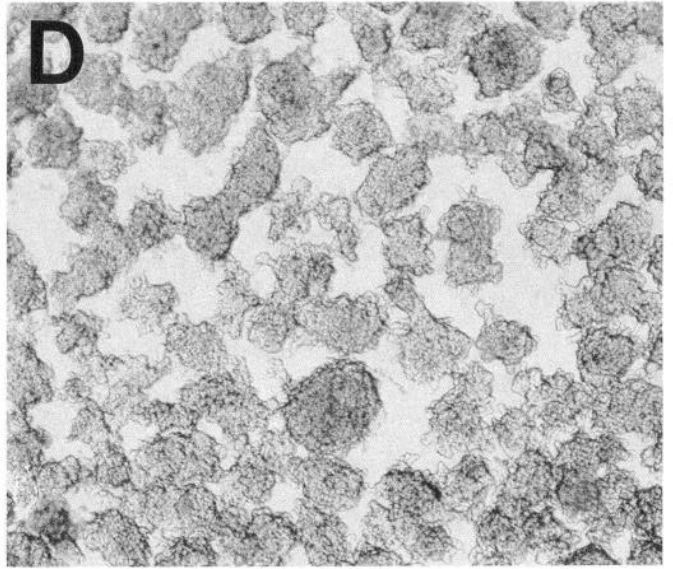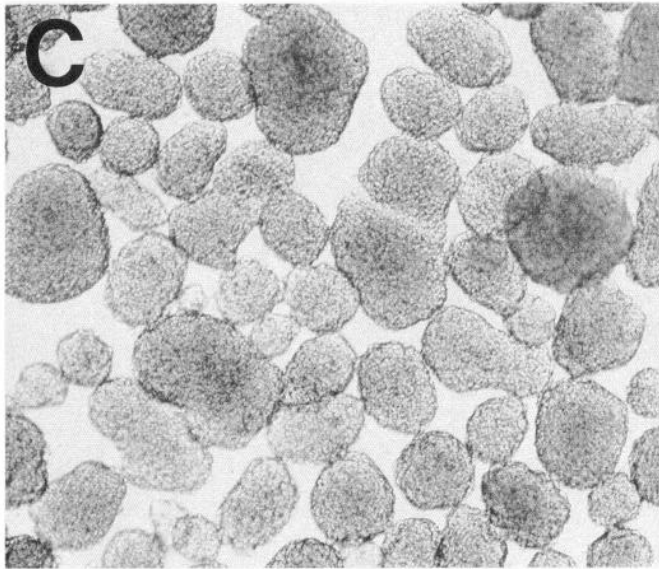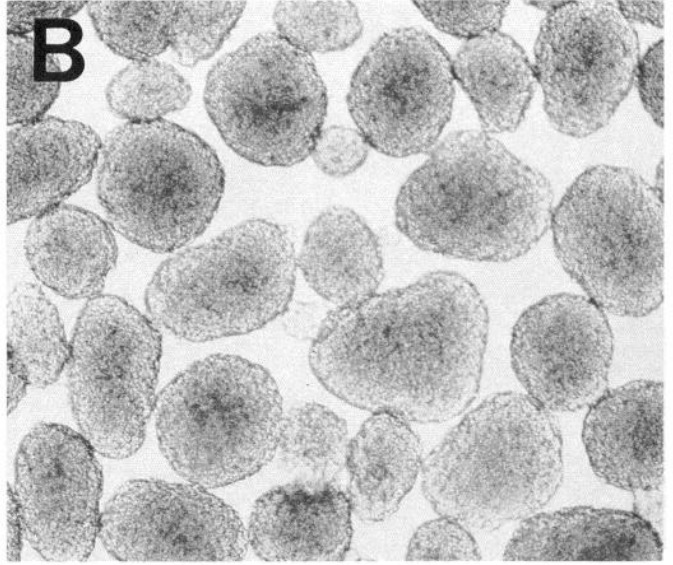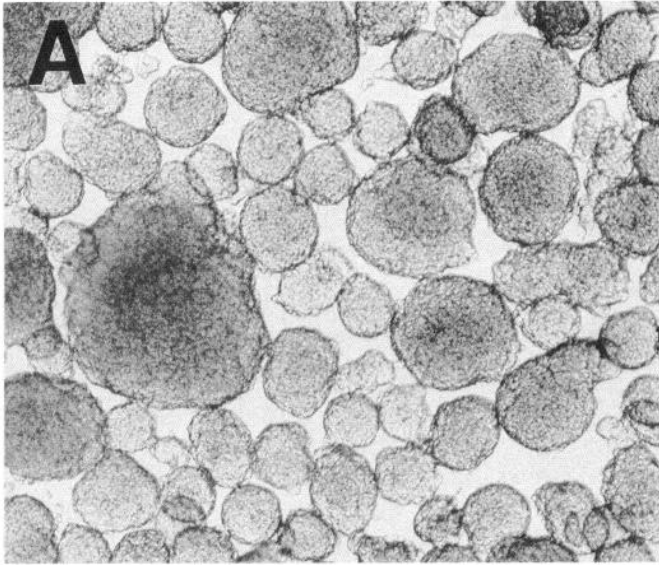
Table 4. D_1 and D_2 dopamine receptor-linked modulation of adenylate cyclase activity in monoclonal hybrid corpus striatum cell lines

Assay condition	Cell line					
	N18TG2	X57	X62	X57	X62	X58
Control	7.3 ± 1.6	5.3 ± 2.6	17.4 ± 5.2	10.4 ± 2.9	8.8 ± 1.3	7.6 ± 4.5
SKF38393	6.5 ± 1.7	6.8 ± 3.7[f]	24.5 ± 5.9[a]	—	—	—
SKF38393 + SCH23390	—	5.4 ± 2.9[b]	19.0 ± 5.1[b]	—	—	—
Forskolin	13.0 ± 3.2[a]	—	—	23.6 ± 4.7[a]	28.7 ± 4.8[a]	17.4 ± 7.7[f]
Forskolin + quinpirole	13.9 ± 3.4[a,e]	—	—	24.1 ± 4.5[a,e]	28.8 ± 4.8[a,e]	11.5 ± 5.2[c]
Forskolin + quinpirole + eticlopride	—	—	—	—	—	15.6 ± 6.3[d,e]

Results are expressed as pmol cAMP formed/10 min/mg protein (means ± SEM). In the case of the corpus striatum hybrid cell lines X57 and X62, D_1 receptor-mediated stimulation and D_2 receptor-mediated inhibition of adenylate cyclase activity were examined in separate experiments. Data represent the means ± SEM of at least five separate experiments, each of which served as its own control, and were analyzed by paired sample t test comparison. Assays were performed in triplicate in cell membrane preparations using ^{3}H-ATP as a substrate. The cAMP reaction product was separated by high-pressure liquid chromatography by the method of Childers (1985), and the ^{3}H-cAMP levels determined by liquid scintillation counting. Conditions were Control, no drug; 10 μM SKF38393 (D_1 agonist); 10 μM SKF38393 with 100 μM SCH23390 (D_1 antagonist); 10 μM forskolin; 10 μM forskolin with 1 μM quinpirole (D_2 agonist); and 10 μM forskolin, 1 μM quinpirole with 10 μM eticlopride (D_2 antagonist).

—, not determined.

[a] Values are significantly different from control levels as determined by paired sample t test ($p < 0.01$).

[b] Values are significantly different from SKF38393 alone as determined by paired sample t test ($p < 0.05$).

[c] Values are significantly different from forskolin alone as determined by paired sample t test ($p < 0.05$).

[d] Values are significantly different from forskolin with quinpirole as determined by paired sample t test ($p < 0.05$).

[e] Values are not significantly different from forskolin alone.

[f] Values are significantly different from control as determined by paired sample t test ($p < 0.05$).

phology resembled that of aggregates of primary striatal cells (Fig. 4E), within 36 hr, a finding also observed with dopaminergic hybrid cell lines derived from embryonic murine rostral mesencephalic tegmentum (Choi et al., 1991) and septal cholinergic cell lines (Hammond et al., 1986).

Discussion

Immortalized monoclonal corpus striatum hybrid cell lines possessing properties characteristic of corpus striatum neurons have been generated by fusing embryonic day 18 corpus striatal cells with an HPRT-deficient murine neuroblastoma cell line (N18TG2). The monoclonal cell lines obtained appear to be hybrids of N18TG2 cells and striatal neurons since: (1) the cells survive and multiply in HAT medium, which selects against unfused neuroblastoma cells devoid of the HPRT enzyme; (2) the primary brain cells used as fusion partners are postmitotic and are incapable of multiplication, and for that reason are eliminated in the single-cell cloning procedure; (3) the immortalized cells form aggregates in rotatory culture with a morphology similar to that of primary cells (Fig. 4), while neuroblastoma cells do not share this property; and (4) the hybrid cells elaborate neurite-like processes in the presence of n-butyric acid or forskolin, which react to the neurofilament protein-specific antibody 4.3F₉ (Fig. 3). There was no detectable labeling of these processes under control or differentiating conditions

with an antibody directed against glial fibrillary acidic protein. Under the same conditions, no immunoreactivity for NFP or GFAP was observed in the N18TG2 cells.

The monoclonal cell lines express an array of D_1, D_2, D_4, and D_5 dopamine receptor mRNA in various combinations (Table 3). The majority of these lines also express varying amounts of mRNA for the medium spiny neuron D_1 receptor-specific marker DARPP-32. Of the lines selected for more detailed characterization, two (X57 and X62) were demonstrated to have functional D_1 receptors and one line (X58) had functional D_2 receptors. While all four of the lines expressed ChAT activity, a feature characteristic of large corpus striatum interneurons, only two of the lines (X52 and X58, Table 2) actually synthesized detectable amounts of acetylcholine.

While these hybrid cell lines express a variety of neurochemical markers characteristic of striatal neurons, it is not possible to identify a single neuronal parent for each of the monoclonal lines. The majority of the neuronal population of the corpus striatum is comprised of medium spiny neurons, which are the principal output cells of the corpus striatum and contain GABA (Graybiel and Ragsdale, 1983; Gerfen et al., 1987; Gerfen, 1988, 1992; Graybiel, 1990). The anatomic and functional compartmentalization of these cells has been defined by their afferent projections (to the globus pallidus and substantia nigra pars reticulata) and their neurochemistry. The GABAergic medium

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Figure 3. Photomicrographs of selected corpus striatum monoclonal hybrid cell lines stained immunocytochemically for neurofilament protein (NFP). A, Undifferentiated X57 hybrid cells. B, Undifferentiated X58 hybrid cells. C, Undifferentiated X62 hybrid cells. D, X57 hybrid cells differentiated with forskolin. E, X57 hybrid cells differentiated with n-butyric acid. F, Undifferentiated N18TG2 cells. Differential interference optics were used to visualize the unstained somata and processes that were not well visualized under bright-field optics. Note the absence of NFP staining in the N18TG2 neuroblastoma (F) compared to the hybrid cells (A–E). Both the hybrid cell lines X58 and X62, but not the N18TG2 cells, exhibited similar morphological responses (extension of neurite-like processes) and NFP immunoreactivity when differentiated under the same treatment conditions. Scale bar, 100 μm.
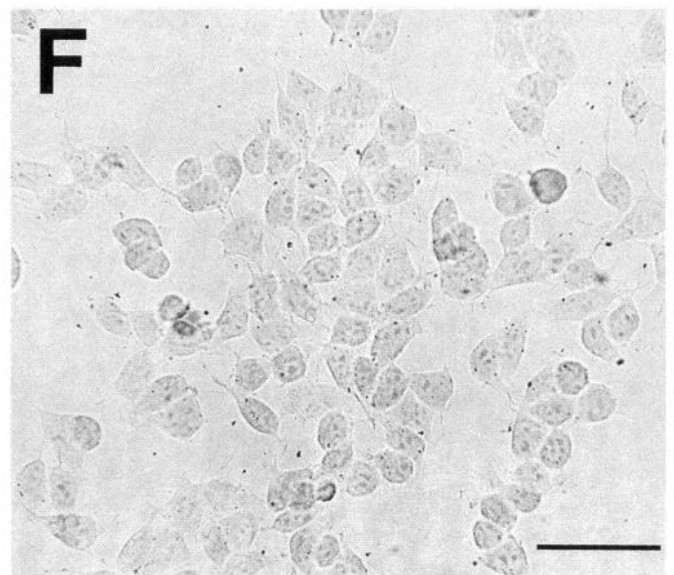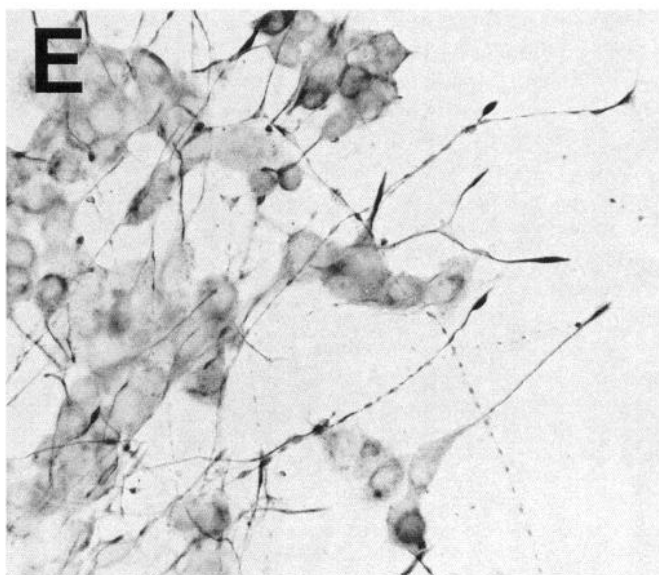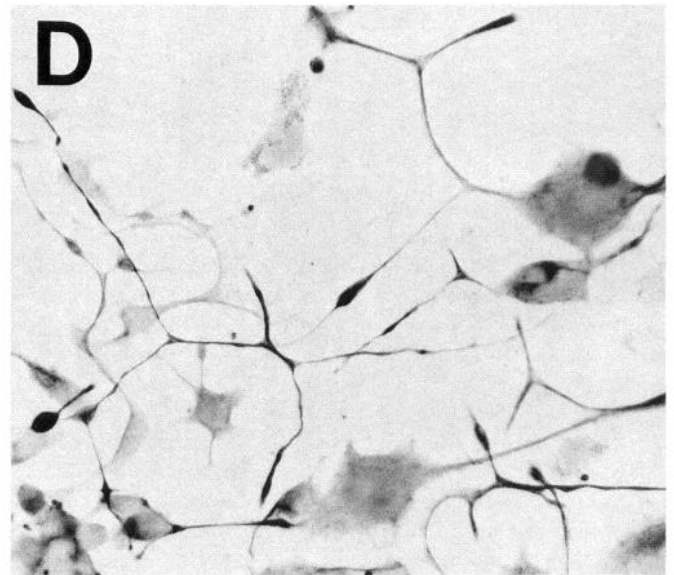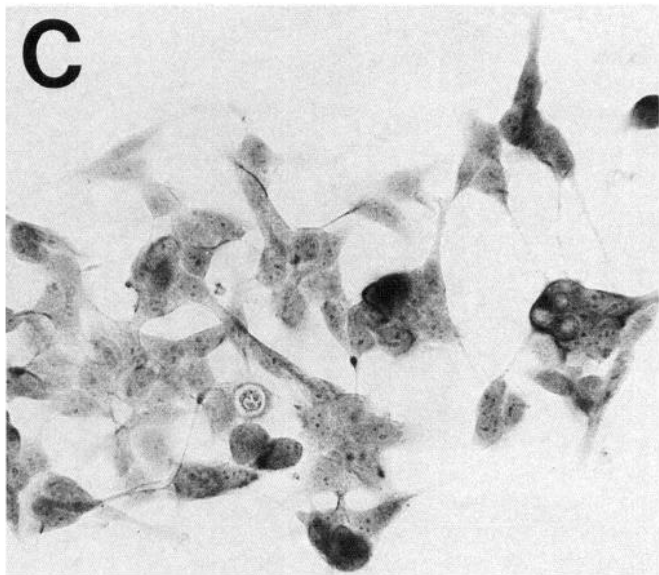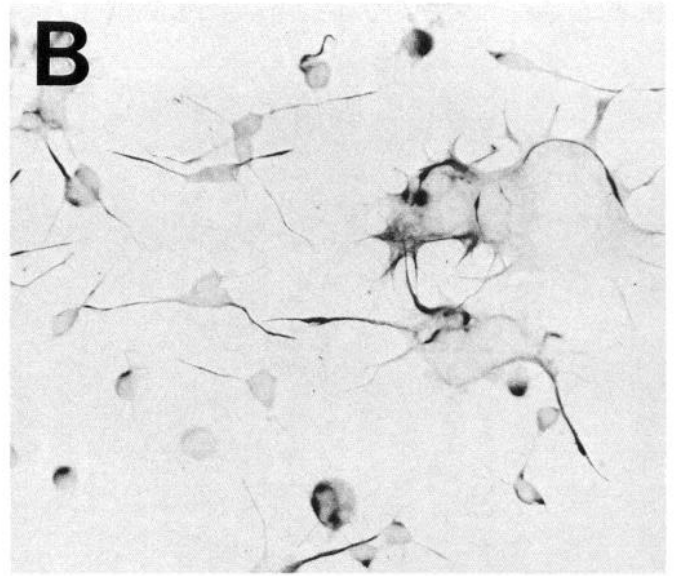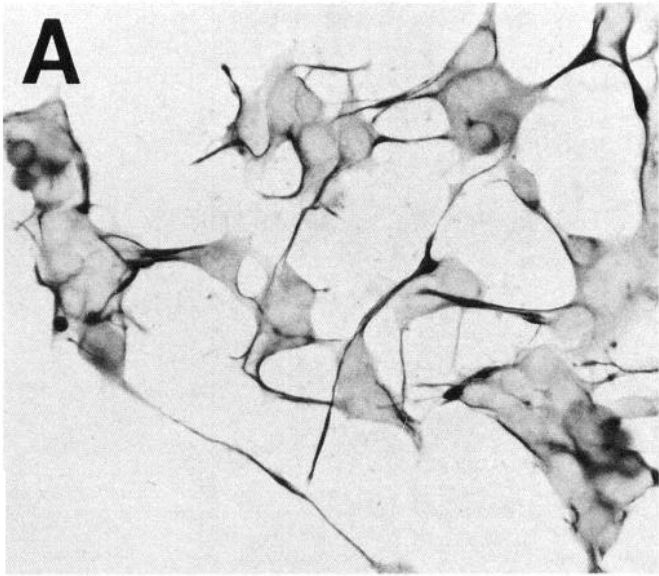
spiny neuron population accounts for approximately 90% of the cell population (Kita and Kitai, 1988). The remainder of the neuronal population within the corpus striatum is comprised of larger neurons. These include large, aspiny cholinergic interneurons as well as some medium aspiny neurons which contain GABA (Bolam et al., 1985) or somatostatin (DiFiglia and Aronin, 1982).

The precise distribution of dopamine receptor subtypes within corpus striatum neuronal populations has not yet been resolved (Gerfen, 1992; Surmeier et al., 1993). D_1 receptors have been localized principally to dynorphin and substance P–containing striatonigral medium spiny neurons (Gerfen et al., 1990; Le Moine et al., 1990), and possibly some cholinergic interneurons (Bernard et al., 1992). D_2 receptors are present on striatopallidal, enkephalinergic medium spiny neurons and large cholinergic interneurons, as well as in a noncholinergic neuronal population (Fink et al., 1992). Several lines of evidence suggest that D_1 and D_2 receptors may be coexpressed within the same corpus striatum neuron. Studies of the synergistic interactions between D_1 and D_2 receptors with respect to $(Na^+ + K^+)$ ATPase activity (Bertorello et al., 1990) have suggested that these receptors may be coexpressed in the same cells. Using RT-PCR techniques, Surmeier et al. (1992) have described the coexpression of D_1, D_2, and D_3 cDNA in single striatal neurons. *In situ* hybridization studies (Lester et al., 1993) indicate that D_1 and D_2 receptor mRNA may be coexpressed in approximately 26% of the neurons of the corpus striatum. Therefore, the neuronal parent of a monoclonal hybrid cell line cannot be identified by the expression of a specific dopamine receptor.

The nature of the fusion process, itself, is such that the cell lines obtained are heterogenous in nature. It is likely that chromosomes from more than one primary cell type may be incorporated in the final fusion product and that chromosomes are also lost in the fusion process (Killary and Fournier, 1984). The monoclonal hybrid cell lines described here in all probability contain an admixture of chromosomes resulting from the fusion of one or more primary corpus striatum cells with the neuroblastoma. The somatic cell fusion process may capture either components of biochemically disparate cell types or partially capture components of a single cell type. For example, we have described (Choi et al., 1991) a hybrid cell line, MN9D, which produces and stores dopamine. Another line, MN9H, produced in the same fusion, synthesizes dopamine but apparently lacks adequate storage mechanisms for the neurotransmitter, probably due to a failure to incorporate necessary chromosomal material into the final fusion product. Given these considerations, it is clearly not possible to ascribe all of the characteristics of a given hybrid cell line to the exclusive participation of any particular subset of striatal neurons in the fusion process. Nevertheless, considering the expression of specific neurochemical markers, including ChAT, ACh, dopamine receptors, and DARPP-32 mRNA in the immortalized monoclonal hybrid cell lines, it is probable that corpus striatum medium spiny neurons and cholinergic interneurons participated in the fusion process.

It is interesting that despite the preponderance of GABA-expressing medium spiny neurons in the corpus striatum, no GABAergic hybrid cell lines were generated from the fusion process. It seems likely that medium spiny neurons did participate in the fusion process, given the large numbers of cells of this type in the striatum and the presence of mRNA for DARPP-32, a marker for such cells, in many of the monoclonal hybrid cell lines. However, since the hybrid cell lines are subclones from a small population of cells which survived treatment with n-butyric acid and a specific D_1 agonist, it is possible that the lines were obtained from corpus striatum cells which, in fact, did not express GABA. Another possibility is that the fusion process itself may have either excluded chromosomal material necessary for GABA synthesis or repressed the expression of the GABAergic phenotype of medium spiny neurons which participated in the fusion.

In contrast to the absence of GABA, substantial levels of ChAT activity were detected in the cell population (E1X) from which the monoclonal hybrid cell lines were derived. The presence of ChAT activity and endogenous ACh (Table 2) in at least two of these lines (X52 and X58) suggests that some of these cell lines are derived, at least in part, from the cholinergic corpus striatum interneuron. The sixfold difference in ChAT activity was not correlated with the difference in ACh content (greater than 20-fold) between the two cell lines. It is unlikely that the high levels of ChAT activity and the ability to synthesize ACh are derived from genes that are silent in the N18TG2 cell line (Mevel-Minio and Weiss, 1981; Killary and Fournier, 1984). No ChAT activity was detected in a mesencephalic dopaminergic cell line (MN9D) produced with N18TG2 as the fusion partner (Choi et al., 1992). On the other hand, the fusion of N18TG2 cells with primary septal cholinergic cells resulted in cell lines which do express ChAT activity (Hammond et al., 1986, 1990; Lee et al., 1990a), suggesting that the expression of a cholinergic phenotype in cell lines produced by the somatic cell fusion method is most likely a function of the lineage of the primary cells participating in the fusion process. In addition, neither ChAT activity nor ACh synthesis was detected in N18TG2 cells grown in basal medium or under differentiating conditions as reported by Blusztajn et al. (1992). Thus, the expression of ChAT activity and ACh synthesis in the hybrid cells, together with K^+-evoked ACh release from X52 cells (Wainwright et al., 1993), most likely reflects the expression of C57BL/6J genes from cholinergic interneurons of the corpus striatum.

The caudate-putamen contains the highest levels of DARPP-32 phosphoprotein and DARPP-32 mRNA in the brain (Ehrlich et al., 1990; Schalling et al., 1990). Within the caudate, over 80% of the medium spiny neurons contain DARPP-32 (Hemmings and Greengard, 1986; Ehrlich et al., 1990; Ouimet and Greengard, 1990). While DARPP-32-immunoreactive cells can be demonstrated by E14 in the neostriatum (Foster et al., 1987), no mRNA is detectable prior to birth either by *in situ* hybridization techniques (Gustafson et al., 1992) or by Northern blot analyses (Ehrlich et al., 1990). *In situ* hybridization studies (Gustafson et al., 1992) indicate that DARPP-32 is not expressed in

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Figure 4. Aggregate formation in the three-dimensional reaggregate culture system. Aggregates were photographed 36 hr after the initial aggregation. *A*, X57 hybrid cells. *B*, X58 hybrid cells. *C*, X62 hybrid cells. *D*, N18TG2 cells. *E*, Primary embryonic day 14 striatal cells after 21 d in culture. Note the well defined morphology of the aggregates formed by hybrid cell lines (*A, B, C*) and primary striatal cells (*E*) compared to the irregular clumping observed in the N18TG2 cells (*D*). Scale bar, 500 μm.

cholinergic corpus striatum neurons. Therefore, the detection of DARPP-32 mRNA by RT-PCR techniques in the majority of monoclonal cell lines obtained (Table 3) would suggest that the medium spiny neurons of the striatum were probably frequent participants in the initial cell fusion process.

The demonstration of functional D_1 (X57 and X62) and D_2 receptors (X58) coupled to the modulation of adenylate cyclase activity in the hybrid cell lines, but not the N18TG2 cells, suggests that the expression of functional dopamine receptors in the hybrid cells is also a property derived from the corpus striatum neuronal parent cells. No dopamine receptor mRNA, except for a trace of D_4 transcript, was detected in the N18TG2 cells. Further, we have not detected mRNA for any of the dopamine receptor subtypes in the dopaminergic cell line MN9D (Choi et al., 1991) derived from the fusion of N18TG2 cells with embryonic mouse mesencephalon (Tang et al., 1994). The density of D_1 and D_2 binding sites in the hybrid cell lines described in this report (less than 50 fmol/mg protein) is low compared to the adult rodent corpus striatum (2000 ± 183 fmol/mg protein) as reported, for example, by Gelbard et al. (1989) (for review see Vadasz et al., 1992). However, the gestational age of the primary corpus striatum cells from which these hybrid lines were derived (E18) precedes the onset of the rapid, postnatal increase in D_1 and D_2 receptor density observed in the rodent corpus striatum (Bruinink et al., 1983; Murrin and Zeng, 1986, 1990; Zeng et al., 1988; Gelbard et al., 1989; Rao et al., 1991) as well as the robust increase in structure and activity of dopaminergic synapses that occurs over the first four postnatal weeks (McGeer et al., 1971; Coyle and Axelrod, 1972; Porcher and Heller, 1972).

The extent of stimulation of adenylate cyclase seen in cell lines X57 and X62 in the presence of a D_1 agonist and forskolin is also modest. There are a number of possible explanations for this observation. A modest stimulation of adenylate cyclase activity, despite the presence of a high density of receptors, is a distinctive feature of adult striatal D_1 receptor pharmacology (Battaglia et al., 1986; Mailman et al., 1986). More specifically, the monoclonal hybrid cell lines are derived from embryonic day 18 corpus striatum neurons and, therefore, may not be directly comparable to adult corpus striatum neurons with respect to dopamine receptor-mediated modulation of adenylate cyclase activity. Indeed, the twofold increase in adenylate cyclase activity in response to forskolin stimulation in the corpus striatum hybrid cell lines is comparable to that reported in other studies (Chneiweiss et al., 1988) involving embryonic murine striatal membrane preparations. While the partial D_1 receptor agonist SKF 38393 has been widely used in studies reporting the expression of functional, cloned D_1-like receptors (Dearry et al., 1990; Zhou et al., 1990; Tiberi et al., 1991), the efficacy of SKF 38393 in the examination of D_1 receptor-mediated stimulation of adenylate cyclase activity in the monoclonal hybrid cell lines may be limited by the low density of receptors in the cell lines examined. It is possible that the use of a full agonist such as fenoldopam may lead to greater expression of receptor-mediated stimulation of adenylate cyclase activity.

The availability of immortalized monoclonal hybrid neuronal cell lines of striatal origin provides a stable, homogenous cell population for the study of corpus striatum neurochemical properties. While cell lines from other sources (Balmforth et al., 1986, 1988; Monsma et al., 1989; Sidhu and Fishman, 1990; Ivins et al., 1991; Lovenberg et al., 1991; Steffey et al., 1991) provide models in which to examine aspects of dopamine receptor bio-

chemistry and pharmacology, they are not derived from the corpus striatum and, therefore, may or may not be relevant for assessing the regulation of receptor effector systems in this brain region. In this regard, a series of recent studies has demonstrated the importance of cell lineage in the use of cell lines as models from specific brain regions. First, the mesencephalic dopaminergic cell line MN9D is much more sensitive than PC12 cells to the cytotoxic effects of the dopaminergic neurotoxin MPP^+, although both cell lines contain dopamine and accumulate similar levels of MPP^+ (Choi et al., 1991). Second, in a comparative study of D_2-like receptor pharmacology and function, transfected D_4 receptors have been shown to be linked to the modulation of adenylate cyclase activity in the hybrid MN9D cells, but not in the fibroblast cell line CCL1.3 (Tang et al., 1994). Third, the MN9D cells, in contrast to PC12 cells, have been shown in reaggregate cultures with primary cells to be capable of responding in a differential fashion to dopaminergic target and nontarget cells (Choi et al., 1992). The MN9D cell line shows a marked reduction in its dopaminergic phenotype when coaggregated with nontarget cells such as optic tectum or thalamus. The specificity of these effects underscores both the potential utility of mesencephalic and corpus striatal cell lines as well as the importance of the specific cellular milieu in providing an appropriate environment for facilitating the expression of brain region–specific properties. The corpus striatal monoclonal hybrid cell lines described in this report recapitulate the biochemistry of primary corpus striatum neurons and their region-specific lineage and should provide appropriate models for a variety of studies on the neurochemistry of striatal neurons and the role of cell–cell interactions in the regulation of striatal function.

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