Endocrine, vol. 4, no. 1, 35–42, February 1996 0969-711X/96/4:35-42/$7.00

GFAP Expression Induced by Dopamine D_2 Receptor Agonists in the Rat Pituitary Intermediate Lobe

Scott A. Sands and Bibie M. Chronwall

School of Biological Sciences, University of Missouri–Kansas City, Kansas City, MO

This study was conducted to determine if intermediate lobe glial-like cells are affected by compounds that regulate melanotrope biosynthetic activity via the dopamine D_2 receptor. Glial-like cells were stellate, and expressed glial fibrillary acidic protein (GFAP) and vimentin in cell bodies and processes as revealed by immunohistochemistry. Following bromocriptine and quinpirole treatments, the number of cell bodies and processes expressing vimentin did not change, whereas those expressing GFAP increased, although never to exceed the number of vimentin containing structures. The percent of cells and processes coexpressing GFAP and vimentin also increased. The GFAP response was reversible by haloperidol treatment following the agonist treatment. Haloperidol treatment alone did not change GFAP expression. Thus, following D_2 receptor agonist treatment, GFAP was induced in pre-existing vimentin-positive glial cells. Dopamine D_2 receptor mRNA and protein were detected in melanotropes, but not in cells expressing GFAP or vimentin. Although glial-like cells may express dopamine D_2 receptor mRNA and protein below the detection levels of our methods, the possibility of an indirect effect via dopamine D_2 receptor agonists acting on melanotropes needs to be considered.

Introduction

Research in the rat pituitary intermediate lobe has centered on melanotropes, the polyhedral endocrine cells that synthesize proopiomelanocortin (POMC), from which β-endorphin and α-MSH are derived (Eipper and Mains, 1980). A small population of stellate cells of the intermediate lobe, however, does not express POMC, but glial fibrillary acidic protein (GFAP) (Stoeckel et al., 1981; Mudrick-Donnon et al., 1993; Dickerson et al., 1994; Gary and Chronwall, 1995), a specific protein marker of astrocytes in the central nervous system (CNS). Vimentin, an intermediate filament found in CNS glia (Dahl et al., 1981; Schnitzer et al., 1981) is also present in intermediate lobe stellate cells (Sands et al., 1995). These cells are those described in electron microscopic studies as nonglandular interstitial cells containing dark nuclei and cytoplasm protruding between melanotropes (Howe and Maxwell, 1968; Vincent and Kumar, 1969), resembling astrocytes in the CNS.

GFAP immunoreactivity in intermediate lobe glia increases in response to lactation and adrenalectomy (Gary and Chronwall, 1995). In the cerebral cortex, glial cells respond to various forms of stress with extension of their processes and proliferation. This response is known as reactive gliosis. GFAP immunoreactivity (Bignami and Dahl, 1976; Miyake et al., 1988; Janeczko, 1993) and GFAP mRNA (Hozumi et al., 1990) increase in glial cells following stab wounds. MPTP lesions also increase GFAP immunoreactivity (Reinhard et al., 1988; O'Callaghan et al., 1990). An increase in cAMP or the addition of dibutyryl cAMP (Moonen et al., 1975; Sensenbrenner et al., 1980; Goldman and Chiu, 1984), protein kinase C activators (Sawada et al., 1993), IL-1 (Guilian and Lachman, 1985; Balasingam et al., 1994), or IFN-γ (Yong et al., 1991; Balasingam et al., 1994) also increase GFAP immunoreactivity. Glial cells divide following these wounds and lesions (Giulian and Lachman, 1985; Takamiya et al., 1988), but mitosis is not a major factor in the increase in GFAP immunoreactivity (Miyake et al., 1988; Takayima et al., 1988), supporting instead an induction of GFAP in pre-existing astrocytes. Pituicytes, glial cells of the neural lobe, extend their processes around neurosecretory terminals, creating a glial barrier between neurosecretory nerve terminals and capillaries (Tweedle and Hatton, 1980; Hatton et al., 1984; Hatton, 1990). During osmotic stress, pituicytes retract their processes, allowing more contact between terminals and capillaries. This alters extracellular potassium levels, and possibly prevents release of inhibitory substances, such as taurine, normally secreted by pituicytes (Tweedle and Hatton, 1980; Hatton et al., 1984; Hatton, 1990). Stimulation of pituicyte β_2-adrenergic receptors by norepinephrine

Received July 7, 1995; Revised November 2, 1995; Accepted November 20, 1995.

Author to whom all correspondence and reprint requests should be addressed: Bibie M. Chronwall, Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri–Kansas City, 2411 Holmes, Rm M3-CO3, Kansas City, MO 64108.

also results in a retraction of processes (Hatton et al., 1984; Bicknell et al., 1989; Hatton et al., 1991).

We report increased GFAP expression in intermediate lobe glial cells in response to treatments with the dopamine D_2 agonists bromocriptine and quinpirole. This was not owing to an increase in cell proliferation, but to an induction of GFAP in pre-existing cells that shifted intermediate filament expression from vimentin to GFAP. Antagonist treatment following the agonist treatments demonstrated reversibility of the effect. No changes in GFAP immunoreactivity were detected after antagonist treatment alone. Glial cells could respond directly to dopamine, as implied by findings of electrical potentials in glial cells (Mudrick-Donnon et al., 1993). However, neither dopamine D_2 receptor mRNA nor translated protein could be detected by *in situ* hybridization (ISH) and immunohistochemistry. Since melanotrope biosynthesis is regulated by dopamine D_2 receptors, we hypothesize that agonists could have an indirect effect upon glia via melanotropes.

Results

Bromocriptine and Quinpirole Treatments

Vimentin immunoreactivity was localized in filaments in multiple processes and cell bodies of stellate cells (Fig. 1A) in all parts of caudal coronal sections. The number of cells and processes expressing vimentin did not change following bromocriptine (232 ± 13.9; mean ± SEM for control [Fig. 1A], 237 ± 10.0 for bromocriptine) (Fig. 1C) or quinpirole (249 ± 10.9) (Fig. 1E) treatments. The percentage of area covered by immunoreactivity also did not change (13.0 ± 0.92% for control, 13.8 ± 0.53% for bromocriptine, 13.0 ± 0.44% for quinpirole). The pattern of cellular distribution and the morphology of the structures did not change.

GFAP immunoreactivity was also localized in filaments in cell bodies and processes of stellate cells (Fig. 1B). These structures were rarely present in other parts of a caudal coronal section than the dorsolateral regions and nadir of the lobe. The number of immunopositive cell bodies and processes increased significantly following bromocriptine (154 ± 7.0 in control, 227 ± 22.7 in bromocriptine; $p<0.01$) (Fig. 1D) and quinpirole (261 ± 10.0; $p< 0.001$) (Fig. 1F) treatments. The area covered by GFAP immunoreactive cells and processes increased significantly following bromocriptine (6.62 ± 0.56 for control, 9.66 ± 0.84% for bromocriptine; $p<0.05$) and quinpirole (11.2 ± 0.65; $p<0.001$) treatments. The morphology of cells expressing GFAP did not change. GFAP immunoreactive structures were no longer limited to dorsolateral regions and the nadir but were more evenly distributed over the cross-section of the lobe. The number of cells coexpressing GFAP and vimentin increased following agonist treatments; however, GFAP immunoreactive structures never outnumbered those immunoreactive for vimentin. Vimentin immunoreactive intensity decreased in this region (Fig. 1C,E), whereas that of GFAP appeared increased (Fig. 1D,F). Evaluation of intermediate lobe sections from rats that had received agonist treatment followed by 5 d of antagonist treatment showed a 40–75% diminution of the number of GFAP immunoreactive structures compared to agonist treatment alone, indicating that the response was reversible.

To determine if microglia were present, immunohistochemistry for OX-42 was performed. As in control rats, OX-42 immunoreactivity was not present in any region of the intermediate lobe, but was present in brain sections processed in parallel with pituitary sections (data not shown).

Haloperidol Treatment

Neither the number of cells and processes immunoreactive for vimentin (232 ± 13.9 for control, 245 ± 16.3 for haloperidol) (Fig. 1G), nor the percentage of area covered by these cells (13.0 ± 0.92% for control, 10.8 ± 0.6% for haloperidol), nor the distribution of vimentin expressing structures changed.

The number of cells and processes expressing GFAP immunoreactivity also did not change (154 ± 7.0 for control, 156 ± 7.6 for haloperidol) (Fig. 1H), nor did the percentage of area covered by GFAP immunoreactivity (5.46 ± 0.41% for control, 6.62 ± 0.56% for haloperidol). Immunoreactive structures remained confined to the dorsolateral regions and the nadir of the sections and the morphology of the cells did not change.

OX-42 immunoreactivity was not present in any region of the lobe, but was present in brain sections processed in parallel with pituitary sections (data not shown).

D_2 Receptor mRNA and Protein Expression in Relation to Glial Protein Immunoreactivity

In control, haloperidol, bromocriptine, and quinpirole treated rats, D_2 receptor mRNA was detected in polyhedral melanotropes, but not in GFAP or vimentin immunoreactive stellate shaped cells in the same section (Fig. 2A–D; control and bromocriptine treatment). The identity of melanotropes was further ascertained on adjacent sections, where they were immunoreactive for β-endorphin. When combining the procedures on one section, the ISH was completed first, thus it was not influenced by the immunohistochemical procedure. In sections immunohistochemically double-stained for the dopamine D_2 receptor protein (Fig. 3B,D,F) and vimentin (Fig. 3A) or GFAP (Fig. 3C,E), the detection of the markers was exclusive. The receptor antiserum from Chemicon, designated "D_2/D_3," is directed against the D_2 receptor C-terminus, which is the same as that of the D_3 receptor. Since D_3 receptor mRNA cannot be detected in the pituitary by PCR (Sokoloff et al., 1990), we assumed that in this study the antiserum recognizes the D_2 receptor.

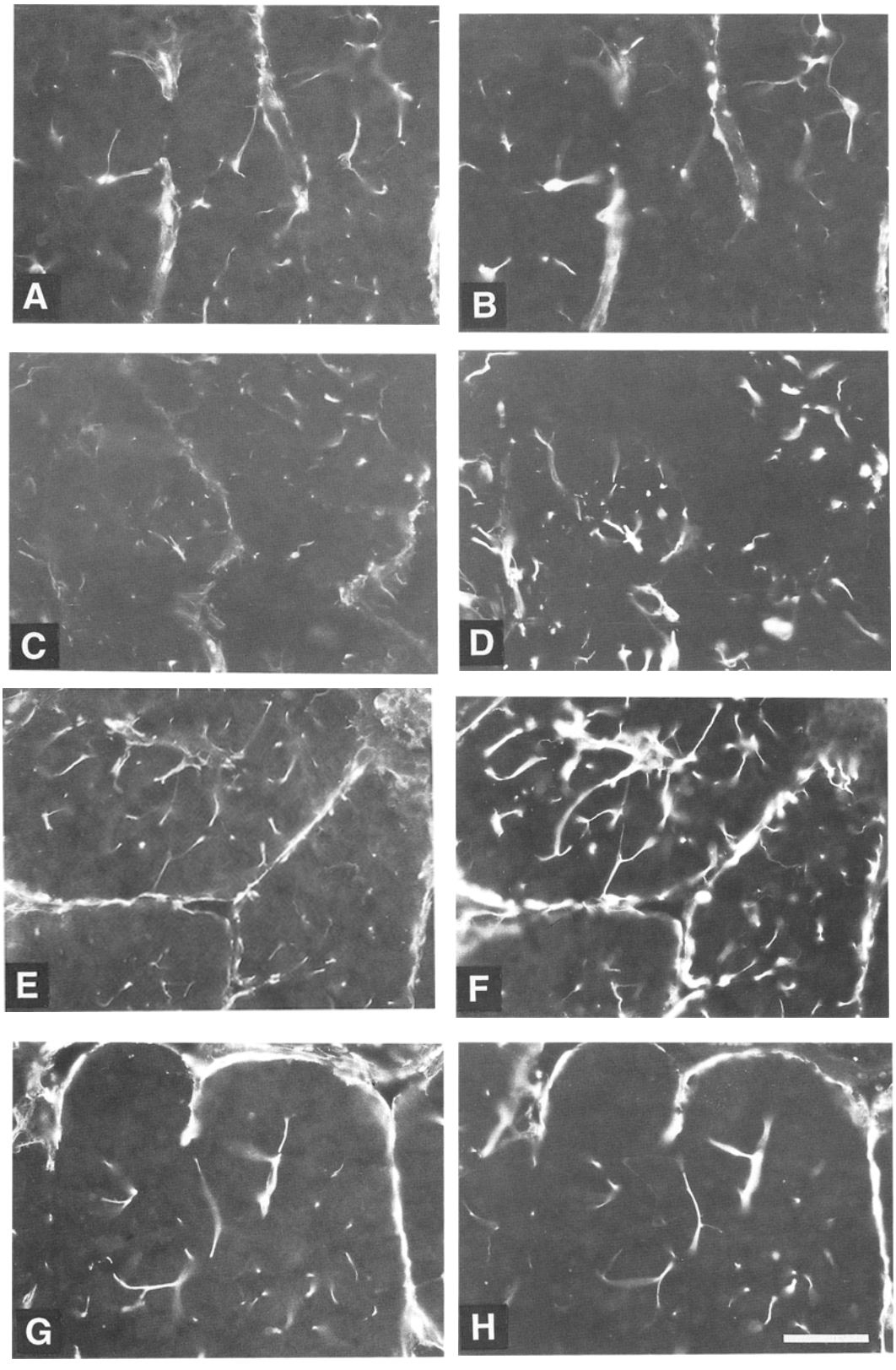

Fig. 1. Double-label immunohistochemistry for vimentin (A,C,E,G) and GFAP (B,D,F,H), demonstrating extensive colocalization of the proteins in pituitary intermediate lobe glial cells from control (A,B); bromocriptine- (C,D); quinpirole- (E,F); and haloperidol (G,H) treated rats. Vimentin is localized in cell bodies and processes of stellate cells, as is GFAP, although fewer cells contain GFAP. The number of cell bodies and processes expressing vimentin did not change following bromocriptine (C); quinpirole (E) or haloperidol (G) treatments, while the number of cell bodies and processes expressing GFAP increased following bromocriptine (D) and quinpirole (F) treatments. No change was demonstrated following haloperidol treatment (H). The photographs are oriented with the dorsal aspect of the lobe at the top; the neural lobe is only visible in the top right corner of (E) and (F) and at the very top of (G) and (H). Bar 50 μm; magnification × 265.

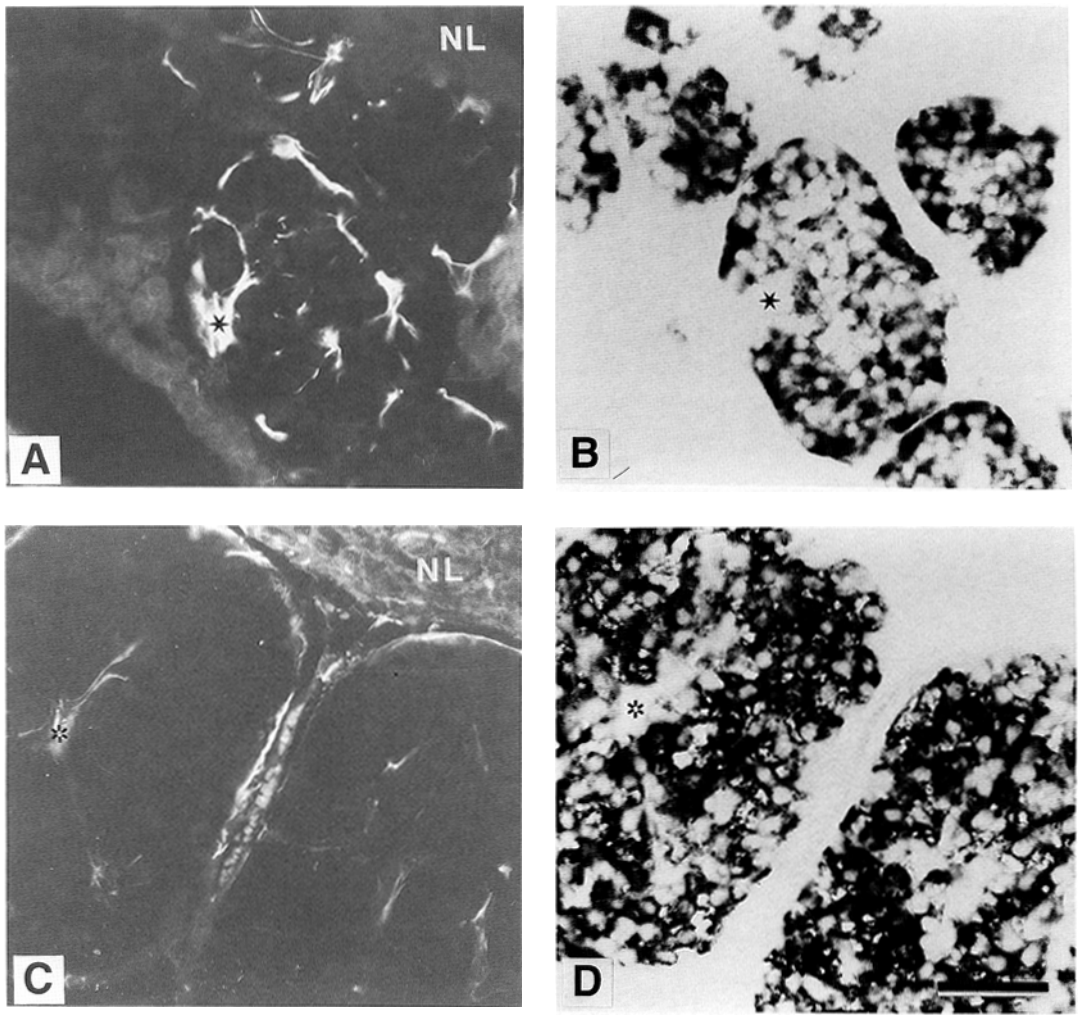

Fig. 2. Combined immunohistochemistry and *in situ* hybridization histochemistry for GFAP (A) and dopamine D_2 receptor mRNA (B) on an intermediate lobe section from a control rat; and vimentin (C) and D_2 receptor mRNA (D) on a section from a bromocriptine treated rat. A comparison of the fluorescence immunoreactive areas, bright areas in (A) and (C), with the precipitate indicating hybridization, dark areas in (B) and (D), demonstrates the complementarity of the labels. No dopamine D_2 receptor mRNA could be detected in the glial cells (examples indicated by stars and asterisks). Bar 50 μm; magnification × 290.

Discussion

Since glial cells in the intermediate lobe respond to changes in physiological state like adrenalectomy, lactation, and salt-loading (Gary and Chronwall, 1995), we have tested the hypothesis that treatments known to cause changes in melanotrope biosynthetic activity could also influence the glia. The route for this influence could be direct through receptor stimulation or indirect through a dynamic relationship between melanotropes and glia.

Melanotrope biosynthetic activity is regulated by dopamine through the D_2 receptor, which is negatively linked to adenylate cyclase (Cote et al., 1982). Antagonists to the D_2 receptor increase POMC gene expression (Höllt et al., 1982; Chen et al., 1983; Chronwall et al., 1987; Loeffler et al., 1988) and α-MSH levels (Meunier and Labrie, 1982). Agonists produce the opposite response (Beaulieu et al., 1984; Chronwall et al., 1987; Levy and Lightman, 1988), which can be reversed by an antagonist (Beaulieu et al., 1984). We employed the antagonist haloperidol, and the agonists bromocriptine and quinpirole, to alter melanotrope activity, and we examined glial cell morphology and intermediate filament expression. An induction of GFAP expression and a possible shift from vimentin to GFAP expression occurred rather than a proliferation of GFAP-expressing cells, since the percent area and number of GFAP immunoreactive cells did not surpass those positive for vimentin. The glial response was reversible when haloperidol treatment followed the agonist treatment.

With glial-like cells responding to dopamine D_2 receptor agonists, we wanted to determine whether or not receptor protein or mRNA could be detected in glial cells. Dopamine binding sites are found in cultured astrocytes from the striatum (Hösli and Hösli, 1986), as are D_1 and D_2 receptor mRNAs (Bal et al., 1994). Neither D_2 receptor mRNA nor dopamine binding sites are detected in neural lobe pituicytes using ISH (Mengod et al., 1989; Chronwall

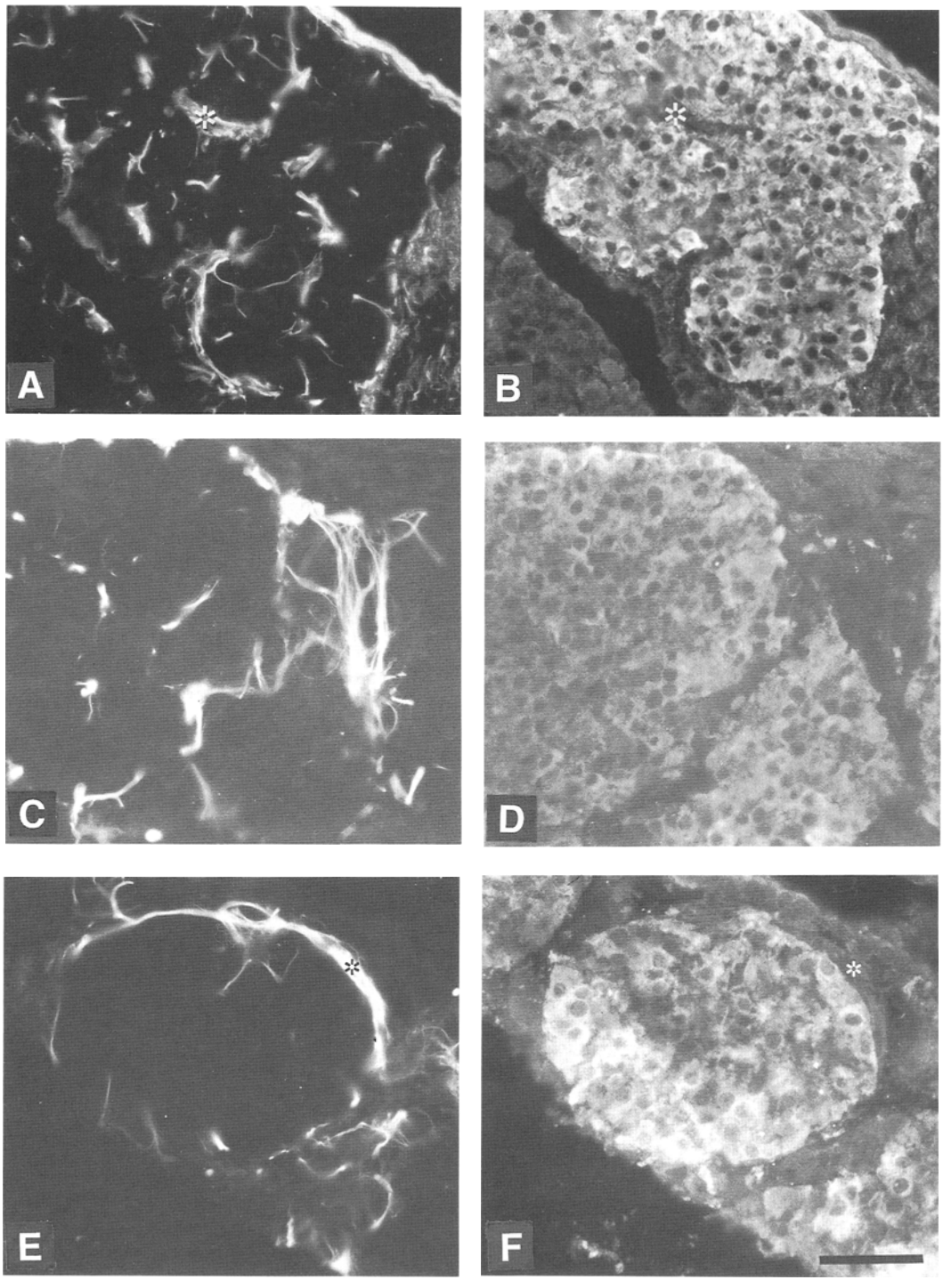

Fig. 3. Double-label immunohistochemical localization of vimentin (A) and GFAP (C, E) and dopamine D_2 receptor (B, D, and F) in a control section (A, B) and two sections from bromocriptine treated animals (C–F). Comparison of cells marked with stars in (A) and (E) and the large aggregate of glial cells in (C) with areas lacking immunoreactivity for the dopamine D_2 receptor protein, demonstrate that neither combination detected dopamine D_2 receptor protein in a cell containing intermediate filament protein. The photographs are dominated by part of a lobule, roughly indicated by the immunoreactive area in B, D, and F; melanotrope nuclei are visible as nonimmunoreactive areas within each cell. Bar 50 μm; magnification × 300.

et al., 1994) or ligand binding (Lightman et al., 1982; Pazos et al., 1985). We detected D_2 receptor mRNA in melanotropes as identified by their shape and immunoreactivity for β-endorphin, but not in cells immunoreactive for GFAP and vimentin. Instead, these cells were interposed between cells expressing D_2 receptor mRNA. Likewise, we could not detect the receptor protein in glial-like cells. Although both mRNA and protein may be present below the detection level of our methods, the possibility remains that these glia may not respond directly by receptor stimulation, but

indirectly through interactions with melanotropes. On the other hand, bromocriptine and quinpirole could be functioning in other ways than as receptor agonists. While little is known about quinpirole beyond its role as a D_2 agonist, bromocriptine causes apoptotic cell death in lactotropes (Drewett et al., 1993), which possess the D_2 receptor. In prolactin-producing adenomas, bromocriptine treatment results in an involuted cell nucleus and coarse clumping of the chromatin (Tindall et al., 1982), characteristics commonly associated with apoptotic cells. ATP may be released from apoptotic cells, which could cause morphological changes in intermediate lobe glial cells and an induction of GFAP expression (Rathbone et al., 1991; Neary et al., 1994). Preliminary data, however, showed no difference between intermediate lobes from control and bromocriptine treated animals in regard to the detection of apoptotic cells.

Since glial cells responded to dopamine agonists, yet not antagonists, glial cells may not interact chemically with melanotropes, but may react to changes in electrical activity of melanotropes. Glial cells did not possess D_2 receptors detectable by our methods, but melanotropes do. Melanotropes spontaneously discharge action potentials (Davis and Hadley, 1976; Douglas and Taraskevich, 1982). Dopamine slows this discharge (Williams et al., 1989), and it is reversed by metoclopramide (Douglas and Taraskevich, 1982), presumably through the D_2 receptor, which, when activated, hyperpolarizes melanotropes (Douglas and Taraskevich, 1978). The change in action potential discharge by dopamine or receptor agonists alters the extracellular sodium and potassium concentration, which can induce changes in glial cells. Glial cells have voltage-dependent potassium currents (Bevan and Raff, 1985) and possess resting potentials indicative of a high permeability to potassium (Kuffler et al., 1966; reviewed in Duffy and MacVicar, 1993). Glial cell function (Orkand and Opava, 1994) and channel activity (Schule and Wuttke, 1983) change with alterations in extracellular potassium concentration. These alterations could cascade to affect GFAP expression. Elevated extracellular potassium is associated with a reduction in extracellular space in the rat neural lobe, with the extension of pituicyte processes playing a key role in this reduction (Hatton, 1990; Armstrong et al., 1991). Elongation of processes may require cytoskeletal reorganization, and perhaps an induction of GFAP expression. Glial cells of the intermediate lobe may be responding to various alterations caused by bromocriptine and quinpirole treatments. Thus, the shift to GFAP expression could be induced by several mechanisms stimulated by dopamine D_2 receptor agonist treatment.

Methods

Animals and Drug Treatments

Male Sprague-Dawley rats (Sasco, Omaha, NE) weighing 150–175 g were housed two to a cage and kept on a 12-h light/dark cycle with food and water available ad libitum. Vehicle (20 m*M* tartaric acid), haloperidol (Sigma; 2.0 mg/kg body wt), quinpirole (Research Biochemicals; 1.0 mg/kg body wt) or 2-bromo-α-ergocryptine (Sigma; 2.0 mg/kg body wt) were administered intraperitoneally at 0830–0900 each day for 14 d. As a control experiment, four animals were treated with either quinpirole (n = 2) or bromocriptine (n = 2) and then received an additional 5 d treatment with haloperidol.

Immunohistochemistry

Six hours after d 14 treatment, the pentobarbital anesthetized rats were decapitated, pituitaries were dissected from the basal cranium and immersion fixed in periodate-lysine-paraformaldehyde (10 m*M* $NaIO_4$, 75 m*M* lysine, 37.5 m*M* $NaPO_4$ buffer, 2% paraformaldehyde) at 4°C overnight. Pituitaries were sunk in 25% sucrose overnight at 4°C, blotted dry, frozen on dry ice, and embedded in OCT compound (Miles, Elkhart, IL). Coronal cryostat sections (14 μm) were thaw mounted on subbed slides. Sections were collected from the caudal third of the pituitary, since GFAP expressing glial cells are restricted to this part of the pituitary (Gary and Chronwall, 1995).

For peroxidase antiperoxidase (PAP) immunohistochemistry, which was used for the quantifications by image analysis, slides were placed in a 4:1 methanol/3% H_2O_2 solution for 5 min, followed by a 15-min incubation in 10% normal goat serum. Slides were then placed overnight in one of the following antisera diluted in phosphate buffered saline with 0.2% Triton X-100 (PBS-TX): rabbit anti-GFAP (1:100, a gift from Lawrence Eng, Veterans Administration Hospital, Stanford, CA), mouse anti-GFAP (1:400, Sigma, St. Louis, MO), mouse antivimentin (1:50, Zymed, South San Francisco, CA), rabbit antidopamine D_2/D_3 receptor (1:500, Chemicon, Temecula, CA), mouse anti-β endorphin (1:50, Boehringer Mannheim, Indianapolis, IN), a melanotrope marker, and mouse anti-OX-42 (1:500, Harlan-Serotec, Indianapolis, IN), a surface marker for cells of mesenchymal origin such as macrophages, phagocytes, and microglia. Pituitary sections from control, haloperidol, bromocriptine, and quinpirole treated rats were processed simultaneously to allow adequate comparison of distribution and intensities of glial protein immunoreactivity. All subsequent steps with the exception of the 3,3-diaminobenzidine (DAB; Sigma) preincubation and DAB reaction were performed at 4°C. Slides were washed two times in 100 m*M* $NaPO_4$ buffer for 15 min each, and incubated for 30 min in a moist chamber with secondary antiserum (goat antirabbit IgG [Cappel] or goat antimouse IgG [Jackson Immunoresearch]) at 1:100 dilution. Slides were washed two times in 100 m*M* $NaPO_4$ buffer for 15 min each, and incubated for 30 min in a moist chamber with PAP conjugate (rabbit PAP [Cappel] or mouse PAP [Jackson Immunoresearch]) diluted 1:100. Slides were washed two times for 15 min each in 100 m*M*

$NaPO_4$ buffer, incubated in 0.05% DAB in 100 m*M* Tris-saline for 10 min and then for 10 min in DAB with 0.001% H_2O_2. The reaction was stopped with PBS. Slides were rinsed with distilled water, air dried, and cover slipped with Permount mounting medium. Incubation in normal serum in PBS-TX instead of primary antiserum was used as a control, with no staining observed.

For fluorescence immunohistochemistry, which was used in combination with ISH and in colocalization determinations, slides were equilibrated for 5 min in PBS-TX and placed in primary antiserum at 4°C overnight. All subsequent steps were performed at room temperature. Slides were washed three times for 5 min each in PBS-TX, and incubated for 1.5 h in a light-sealed moist chamber in the appropriate secondary antiserum (1:300 in PBS-TX), either rhodamine-conjugated goat antirabbit IgG (Cappel) or fluorescein-conjugated goat antimouse IgG (Jackson Immunoresearch). Slides were washed three times for 5 min each in PBS-TX. For double label immunofluorescence, slides were placed in the second primary antiserum overnight, and the procedure was repeated. Slides were cover slipped with 20% Vinol 205 (Air Products and Chemicals, Allentown, PA) in glycerol (1:1 in PBS). No crossreactivity was observed between the first and second secondary antibodies. No staining was observed when the second primary antiserum was omitted in the double label procedure. Changing the order of first and second primary antisera did not alter the results.

In Situ *Hybridization*

The dopamine D_{2Total} receptor oligonucleotide probe, recognizing both isoforms of the receptor and the probe specifically recognizing the dopamine D_{2Long} receptor isoform (Monsma et al., 1989) were used for nonisotopic ISH (Chronwall et al., 1994). The probes were 3'-end labeled with digoxigenin-11-UTP (Genius kit, Boehringer Mannheim, Indianapolis, IN) using terminal transferase at 37°C for 15 min. Sections were rinsed in 2X SSC (300 m*M* NaCl, 30 m*M* sodium citrate) and covered with hybridization buffer (5 mL deionized formamide, 2 mL 20X SSC, 0.2 mL 50X Denhardts, 0.5 mL salmon sperm DNA at 10 mg/mL, 0.25 mL yeast tRNA at 10 mg/mL, and 1.6 mL 50% dextran sulfate) for 1 h at room temperature. The labeled probe (1:200 in hybridization buffer) was pipetted (500 µL) over each slide, and slides incubated overnight at 37°C. Slides were washed with agitation in 2X SSC for 1 h at room temperature, 1X SSC for 1 h at room temperature, 0.5X SSC for 0.5 h at 37°C, and 0.5X SSC for 0.5 h at room temperature.

Slides were rinsed in buffer 1 (100 m*M* Tris-HCl, 150 m*M* NaCl, pH 7.5) for 1 min, and incubated for 30 min at room temperature in 2% normal sheep serum with 0.3% Triton X-100 in buffer 1. Sheep antidigoxigenin antibody conjugated to alkaline phosphatase was diluted to a 1:250 working dilution in buffer 1 containing 1% normal sheep serum with 0.3 % Triton X- 100, and 500 µL were pipetted onto each slide. Slides were incubated in a moist chamber at room temperature for 4 h, washed with shaking in buffer 1 for 10 min, and in buffer 2 (100 m*M* Tris-HCl, 100 m*M* NaCl, 50 m*M* $MgCl_2$, pH 9.5) for 10 min. Slides were placed in a moist, light-tight humid chamber at room temperature with 500 µL per slide of color solution (nitroblue tetrazolium [NBT] and X-phosphate in dimethylformamide with levamisole) overnight (16–20 h). Color reaction was stopped with buffer 3 (10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0). Sections were dehydrated and cover slipped with Permount. Controls included the omission of the probe or the antidigoxigenin antibody, with no staining observed in either case. Sections of the striatum were used as a positive control; hybridization was seen in neurons as previously demonstrated (Mengod et al., 1989; Monsma et al., 1989). A nonsense probe did not hybridize. The similar distribution of receptor mRNA and protein seen in this study serves as an additional control.

For the immunohistochemistry and ISH combination, the steps were performed as described above to the 30 min incubation in buffer 1 with 2% normal sheep serum and 0.3% Triton X-100. Slides were then placed in primary antiserum overnight, and fluorescence immunohistochemistry was performed as described above. After a rinse in distilled water, slides were placed in the sheep antidigoxigenin antibody conjugated to alkaline phosphatase diluted to a 1:250 working dilution in buffer 1 containing 1% normal sheep serum with 0.3% Triton X-100, with 500 µL pipetted onto each slide and processed as described. Adjacent sections processed using single label immunohistochemistry showed similar immunohistochemical staining as the double label preparations.

Quantification

The number of structures expressing GFAP or vimentin immunoreactivity, representing glial cell bodies as well as processes, were manually counted in PAP preparations from three entire sections from three animals from each treatment. The percent area of the lobe covered by GFAP or vimentin immunoreactivity was calculated using an image analysis system consisting of a Dage/MTI model 72 CCD camera mounted on the trinocular port of an Zeiss Axioplan microscope. The camera was connected to a Matrox MVP-AT array processor installed in a 486-based AT bus PC with 80486 math coprocessor, 16 MB RAM, 190 MB hard disk drive, and running image processing and analysis software (Micro Measure FL-4000, Georgia Instruments, Roswell, GA). Three coronal sections from comparable levels of the caudal third of the intermediate lobe, where GFAP immunoreactivity was present, from three different animals from each treatment were evaluated. All values were expressed as mean ± SEM and were evaluated by one-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test (GraphPad InStat, San Diego, CA).

Acknowledgments

This research was supported by NIH Grant NS28019 to BMC. The authors thank Dr. William R. Millington for his critical review of the manuscript.

References

Armstrong, W. A., Tian, M., and Reger, J. F. (1991). *J. Neurocytol.* **20,** 364–372.

Bal, A., Bachelot, T., Savasta, M., Manier, M., Verna, J. M., Benabid, A. L., and Feuerstein, C. (1994). *Mol. Brain Res.* **23,** 204–212.

Balasingam, V., Tejada-Berges, T., Wright, E., Bouckova, R., and Yong, V. W. (1994). *J. Neurosci.* **14,** 846–856.

Beaulieu, M., Goldman, M. E., Miyazaki, K., Frey, E. A., Eskay, R. L., Kebabian, J. W., and Cote, T. E. (1984). *Endocrinol.* **114,** 1871–1884.

Bevan, S. and Raff, M. (1985). *Nature* **315,** 229–232.

Bicknell, R. J., Luckman, S. M., Inenaga, K., Mason, W. T., and Hatton, G. I. (1989). *Brain Res. Bull.* **22,** 379–388.

Bignami, A. and Dahl, D. *Neuropath. Appl. Neurobiol.* **2,** 99–110.

Chen, C. L. C., Dionne, F. T., and Roberts, J. L. (1983). *Proc. Natl. Acad. Sci. USA* **80,** 2211–2215.

Chiu, F. C. and Goldman, J. E. (1985). *J. Neuroimmunol.* **8,** 283–292.

Chronwall, B. M., Dickerson, D. S., Huerter, B. S., Sibley, D. R., and Millington, W. R. (1994). *Mol. Cell. Neurosci.* **5,** 35–45.

Chronwall, B. M., Millington, W. R., Griffin, W. S. T., Unnerstall, J. R., and O'Donohue, T. L. (1987). *Endocrinol.* **120,** 1201–1211.

Cote, T. E., Grewe, C. W., Tsuruta, Z., Stoof, J. C., Eskay, R. L., and Kebabian, J. W. (1982). *Endocrinol.* **110,** 805–811.

Dahl, D., Bignami, A., Wever, K., and Osborn, M. (1981). *Exp. Neurol.* **73,** 496–506.

Davis, M. D. and Hadley, M. E. (1976). *Nature* **261,** 422–423.

Dickerson, D. S., Huerter, B. S., Morris, S. J., and Chronwall, B. M. (1994). *Peptides* **15,** 247–256.

Douglas, W. W. and Taraskevich, P. S. (1978). *J. Physiol.* **285,** 171–184.

Douglas, W. W. and Taraskevich, P. S. (1982). *J. Physiol.* **326,** 201–211.

Drewett, N., Jacobi, J. M., Willgoss, D. A., and Lloyd, H. M. (1993). *Neuroendocrinology* **57,** 89–95.

Duffy, S. and MacVicar, B. A. (1993). In: *Astrocytes, pharmacology, and function* (Murphy, S., ed), Academic, San, Diego, pp. 137–173.

Eipper, B. A. and Mains, R. E. (1980). *Endocr. Rev.* **1,** 1–27.

Gary, K. A. and, Chronwall, B. M. (1995). *Glia* **13,** 272–282.

Goldman, J. E. and, Chiu, F. C. (1984). *Brain Res.* **306,** 85–95.

Guilian, D. and Lachman, L. B. (1985). *Science* **220,** 497–499.

Hatton, G. I. (1990). *Prog. Neurobiol.* **34,** 437–504.

Hatton, G. I., Perlmutter, L. S., Salm, A. K., and Tweedle, C. D. (1984). *Peptides* **5 (Suppl 1),** 121–138.

Hatton, G. I., Luckman, S. M., and Bicknell, R. J. (1991). *Brain Res. Bull.* **26,** 765–769.

Höllt, V., Haarmann, I., Seizinger, B., and Herz, A. (1982). *Endocrinology* **110,** 1885–1891.

Hösli, E. and Hösli, L. (1986). *Neurosci. Lett.* **65,** 177–182.

Howe, A. and Maxwell, D. S. (1968). *Gen. Comp. Endocrinol.* **11,** 169–185.

Hozumi, I., Aquino, D. A., and Norton, W. T. (1990). *Brain Res.* **534,** 291–294.

Janeczko, K. (1993). *Int. J. Devl. Neurosci.* **11,** 139–147.

Kuffler, S. W., Nicholls, J. G., and Orkand, R. K. (1966). *J. Neurophysiol.* **29,** 768–787.

Levy, A. and Lightman, S. L. (1988). *J. Endocrinol.* **118,** 205–210.

Lightman, S. L., Ninkovic, M., and Hunt, S. P. (1982). *Neurosci. Lett.* **32,** 99–102.

Loeffler, J. P., Demeneix, B. A, Kley, N. A., and Höllt, V. (1988). *Neuroendocrinology* **47,** 95–101.

Mengod, G., Martinez-Mir, M. I., Vilaro, M. T., and Palacios, J. M. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 8560–8564.

Meunier, H. and Labrie, F. (1982). *Prog. Neuro. Psychopharmacol. and Biol. Psychiat.* **6,** 411–415.

Miyake, T., Hattori, T., Fukuda, M., Kitamura, T., and Fujita, S. (1988). *Brain Res.* **451,** 133–138.

Monsma, F. J., McVittie, L. D., Gerfen, C. R., Mahan, L. C., and Sibley, D. R. (1989). *Nature* **342,** 926–929.

Moonen, G., Cam, Y., Sensenbrenner, M., and Mandel, P. (1975). *Cell Tiss. Res.* **163,** 365–372.

Mudrick-Donnon, L. A., Williams, P. J., Pittman, Q. J., and MacVicar, B. A. (1993). *J. Neurosci.* **13,** 4660–4668.

Neary, J. T., Baker, L., Jorgensen, S. L., and Norenberg, M. D. (1994). *Acta Neuropathol.* **187,** 8–13.

O'Callaghan, J. P., Miller, D. B., and Reinhard, J. F. Jr. (1990). *Brain Res.* **521,** 73–80.

Orkand, R. K. and Opava, S. C. (1994). *TIPS* **9,** 265–267.

Pazos, A., Stoeckel, M. E., Hindelang, C., and Palacios, J. M. (1985). *Neurosci. Lett.* **59,** 1–7.

Rathbone, M. P., Hindley, S., Gysbers, J. W., and Gabel, B. (1991). *Soc. Neurosci. Abs.* **17,** 550.

Reinhard, J. F. Jr., Miller, D. B., and O'Callaglhan, J. P. (1988). *Neurosci. Lett.* **95,** 246–251.

Sands, S. A., Gary, K. A., and, Chronwall, B. M. (1995). *Int. J. Devl. Neuroscience* **13,** 567–576.

Sawada, M., Suzumura, A., Ohno, K., and Marunouchi, T. (1993). *Brain Res.* **613,** 67–73.

Schnitzer, J., Franke, W. W., and Schachner, M. (1981). *J. Cell. Biol.* **90,** 435–447.

Schule, W. R. and Wuttke, W. (1983). *Brain Res.* **270,** 368–372.

Sensenbrenner, M., Devilliers, G., Bock, E., and Porte, A. (1980). *Differentiation* **17,** 51–61.

Sokoloff, P., Giros, B., Martres, M.-P., Bouthenet, M.-L., and Schwartz, J.-C. (1990). *Nature* **347,** 146–151.

Stoeckel, M. E., Schmitt, G., and Porte, A. (1981). In: *Peptides of the pars intermedia.* Ciba Foundation Symposium, vol. 81, Pittman Medical, London, 101–127.

Takayima, Y., Kohsaka, S., Toya, S., Otani, M., and Tsukada, Y. (1988). *Dev. Brain Res.* **38,** 201–210.

Tindall, G. T., Kovacs, K., Horvath, E., and Thorner, M. O. (1982). *J. Clin. Endocrin. Metab.* **55,** 1178–1183.

Tweedle, C. D. and Hatton, G. I. (1980). *Neuroscience* **5,** 661–667.

Vincent, D. S. and Anand Kumar, T. C. (1969). *Z. Zellforsch* **99,** 185–197.

Williams, P. J., MacVicar, B. A., and Pittman, Q. J. (1989). *Neuroscience* **31,** 673–681.

Yong, V. W., Moumdjian, R., Yong, F. P., Ruijs, T. G. C., Freedman, M. S., Cashman, N., and Antel, J. P. (1991). *Proc. Natl. Acad. Sci. USA* **88,** 7016–7020.