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Endothelin in brain: Receptors, mitogenesis, and biosynthesis in glial cells

(cerebellum/receptor binding sites/inositolphospholipid turnover/vasoconstrictor/peptide/trophic factor)

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ABSTRACT **We have explored the cellular loci of endothelin (ET) actions and formation in the brain, using cerebellar mutant mice as well as primary and continuous cell cultures. A glial role is favored by several observations: (*i*) mutant mice lacking neuronal Purkinje cells display normal ET receptor binding and enhanced stimulation by ET of inositolphospholipid turnover; (*ii*) in weaver mice lacking neuronal granule cells, ET stimulation of inositolphospholipid turnover is not significantly diminished; (*iii*) C_6 glioma cells and primary cultures of cerebellar astroglia exhibit substantial ET receptor binding and ET-induced stimulation of inositolphospholipid turnover; (*iv*) ET promotes mitogenesis of C_6 glioma cells and primary cerebellar astroglia; and (*v*) primary cultures of cerebellar astroglia contain ET mRNA. ET also appears to have a neuronal role, since it stimulates inositolphospholipid turnover in primary cultures of cerebellar granule cells, and ET binding declines in granule cell-deficient mice. Thus, ET can be produced by glia and act upon both glia and neurons in a paracrine fashion.**

Endothelin (ET) is a potent vasoconstricting peptide with multiple isoforms that originally was isolated from vascular endothelial cells (1, 2). In many parts of the body, ET acts on smooth muscle or other cells close to sites of synthesis (3, 4). In peripheral tissues and the brain, ET is a potent stimulator of inositolphospholipid turnover (5). It also stimulates growth of smooth muscle cells and fibroblasts (6, 7).

The sites of synthesis and action of ET in the brain are unclear, though one report describes the *in situ* hybridization and immunohistochemical localization of ET to motor neurons in human spinal cord (8). ET receptor binding sites mapped by autoradiography occur heterogeneously in the brain independently of the distribution of blood vessels, but the cellular elements containing these receptors have not been established (9, 10). In the present study, we use mouse cerebellar mutants and enriched cell populations to demonstrate glial as well as neuronal actions of ET. ET enhances inositolphospholipid turnover within glia, stimulates glial cell growth, and is synthesized within astroglia.

MATERIALS AND METHODS

Materials. ET-1 and ET-3 were purchased from Peptides International (Louisville, KY). ^{125}I-labeled ET-1 (^{125}I-ET-1) was obtained from NEN/DuPont. Male mutant mice with Purkinje cell degeneration (PCD; *pcd/pcd*, C57BL/6J-pcd strain), mice with the nervous mutation (*nr/nr*, C3HeB/FeJ-nr strain), mice with the weaver mutation (*wv/wv*, B6CBA/Ca-A^{W-J}/A-wv strain), and their phenotypically normal littermates were purchased from The Jackson Laboratory. The mice were sacrificed by decapitation 1–3 mo after birth.

Cerebellar primary cell cultures were prepared from 8- to 12-day-old rats as described (11). For the purification of granule cells (12), cells were resuspended in the following culture medium: modified Eagle's medium with D-valine (MEM D-Val; GIBCO) supplemented with 10% (vol/vol) fetal calf serum or dialyzed fetal calf serum (GIBCO), 25 mM KCl, 2 mM glutamine, and 5 mg of gentamicin, 5 mg of kanamycin, and 250 mg of amphotericin B per ml; were plated successively onto 35-mm dishes coated with poly(L-lysine) at 5 μg/ml; and were incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air. Cytosine arabinofuranoside (araC; 10 μM) was added after 24 hr to inhibit the replication of nonneuronal cells. Cells were maintained for up to 2 wk in a similar medium lacking the amphotericin B. Astroglia cultures were purified in a similar way, except cells were initially resuspended in MEM D-Val containing 5 mM KCl, araC was not added, and when confluence was reached, cells were transferred to flasks. Neuronal and astroglial cultures have been characterized by immunocytochemistry and are >90% enriched in their respective cell type; some astroglial cultures used for mRNA analysis contain <0.1% vascular endothelial cells (data not shown).

ET Binding Sites. For binding experiments, ^{125}I-ET-1 (10,000–20,000 cpm; 2200 Ci/mmol; 1 Ci = 37 GBq) was added to tissue or cellular homogenates or to 12-μm fresh-frozen cryostat-cut sections in 50 mM Tris maleate, pH 6.5/150 mM NaCl/1 mM $CaCl_2$/0.1% bovine serum albumin for 1 hr at room temperature (these conditions were found to be optimal for the binding of ^{125}I-ET-1). Homogenates were washed twice with 50 mM NaCl, and radioactivity was measured after rapid filtration (Brandel, Gaithersburg, MD). All binding experiments were carried out within zone A. Because of the slow on-rate, equilibrium could not be conveniently achieved so that K_d and B_{max} values should be viewed as approximations. Sections were washed for two 20-min periods in the incubation buffer, fixed with 4% paraformaldehyde/1% glutaraldehyde for 5 min, dipped quickly in distilled H_2O, and apposed to film or dipped in Kodak NTB2 emulsion and exposed for 1–4 days. Cultured cells were grown in poly(L-lysine)-coated glass slide chambers (Nunc), and binding sites were labeled similarly except in 20 mM Hepes, pH 7.4/150 mM NaCl/1 mM $CaCl_2$/0.1% BSA so as to better preserve cellular integrity; after two 20-min washings in the incubation buffer, cells were wiped from the slides and radioactivity was measured by scintillation spectrometry, or the slides were fixed and viewed as described for tissue sections. In Hepes buffer, ^{125}I-ET-1 was found to bind to homogenates with similar affinity as in the optimal buffer but with slightly lower B_{max}.

Inositolphospholipid Turnover Measurements. Male Sprague–Dawley rats (150–200 g) and mutant and control mice were

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Abbreviations: ET, endothelin; PCD, Purkinje cell degeneration; InsP_x, inositol phosphate(s).

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sacrificed by decapitation; their brains were removed and dissected over ice. Cross-chopped cerebellar slices (350 × 350 μm) were prepared with a McIlwain tissue chopper, dispersed in 15 ml of buffer (123 mM NaCl/5 mM KCl/1.3 mM KH_2PO_4/0.8 mM $CaCl_2$/1.2 mM $MgCl_2$/10 mM glucose/20 mM Hepes, pH 7.4 at 37°C) prewarmed to 37°C, and gassed with 100% O_2 (13). The slices were preincubated at 37°C in a shaking waterbath for 1 hr with two buffer changes.

Inositolphospholipid turnover was estimated by measuring the accumulation of [^{3}H]inositol phosphates ([^{3}H]InsP_x) in the presence of Li^+, which inhibits the enzymatic conversion of inositol 1-phosphate to *myo*-inositol (14). Methods used were almost identical to those published previously (14, 15) except that only 15 μl of gravity-packed tissue slices was added per assay tube and the specific activity of *myo*-[2-^{3}H]inositol was 80–100 Ci/mmol (NEN/DuPont). Proteins were measured with bovine serum albumin as the standard (BCA* Protein Assay Reagent; Pierce).

For assay of inositolphospholipid turnover in tissue-cultured cells, a similar procedure was followed except that cells were previously grown to near confluence within the 24-well tissue culture plates and the medium was changed to Hepes buffer.

Mitogenesis Measurements. Cerebellar primary astroglia or C_6 glioma cells were seeded in 24-well plates and allowed to grow until near confluence. After growth was arrested by transfer to medium lacking fetal calf serum for 24–48 hr, the quiescent cells were stimulated for 24 hr with various concentrations of ET-1 or ET-3 or with 10% fetal calf serum (or with no additions) in medium containing 0.1% bovine serum albumin and 1 μCi of [*methyl*-^{3}H]thymidine (NEN/DuPont; specific activity = 80–90 Ci/mmol) per well, and trichloroacetic acid-insoluble radioactivity was measured (16). In some experiments with the C_6 glioma cells, the cells were stimulated in medium lacking label, and cells were trypsinized from the wells after 24 hr and counted by a Coulter Counter.

RNA Blot-Hybridization (Northern) Analysis. Total RNA (2–20 μg per well) was fractionated in a 1% agarose gel and transferred to nylon membranes. An oligonucleotide probe (39 bases long) complementary to bases 59–97 of the published rat ET-3 sequence (17) was synthesized and purified by HPLC. Of the two ET mRNA species detected by this probe, the higher molecular weight form is most consistent with ET-3, and the lower form, with ET-1 (3). The probe was 3′-end-labeled by using [^{32}P]dATP (5000 Ci/mmol; NEN/DuPont) and terminal deoxynucleotidyltransferase to a specific activity of 4×10^4 Ci/mmol and was incubated overnight with membranes in hybridization buffer containing 50% (vol/vol) formamide and 4× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.4). High-stringency washes the next day were at 55°C in 1× SSC/5% formamide/0.5% sodium dodecyl sulfate for 1 hr. Labeled membranes were apposed to x-ray film (Kodak) for 1–3 weeks.

RESULTS

ET Receptor Binding Sites in Control and Mutant Mice. We examined ^{125}I-ET-1 binding in mutant mice, including two types of Purkinje cell-deficient mutants—the PCD and the nervous strains—both of which lose 85–100% of Purkinje cells during the first weeks of life (18) (Table 1). Neither displayed a significant reduction in numbers of ET-1 binding sites when compared with controls. Weaver mice, which are largely devoid of granule cells (19), displayed a 45% decrease in ET-1 binding per cerebellum when compared with controls. In all control and mutant mice, the K_d for ^{125}I-ET-1 binding was $\approx 8 \times 10^{-10}$ M.

ET Stimulation of Inositolphospholipid Turnover in Control and Mutant Mice. Sokolovsky and collaborators (5) demonstrated potent stimulation of inositolphospholipid turnover by ET in rat cerebellar slices. To ascertain the cellular localization of these effects, we evaluated the neurologic mutant mice (Table 2). For these experiments, ET-3 was used because it is the form whose mRNA predominates in the rat brain (3), though experiments with ET-1 provided similar results (data not shown). In control mice, ET-3 produced a 2- to 3-fold increase of inositolphospholipid turnover. In both forms of mutants deficient in Purkinje cells, the stimulation by ET was substantially greater, with 2.7- and 1.7-fold enhancement compared with the ET-stimulated increases in control mice, respectively. The enhancement of ET effects on inositolphospholipid turnover was not due to denervation supersensitivity in deep cerebellar nuclei neurons postsynaptic to Purkinje cells, since the enhancement was preserved in cerebellar cortex dissected away from deep cerebellar nuclei (data not shown). In weaver mice, which are largely

Table 1. ET receptor binding sites in mutant mouse cerebella

Mutant mouse	Cerebellar weight,* mg	^{125}I-ET-1 B_{max} pmol/mg of protein†	^{125}I-ET-1 B_{max} pmol/cerebellum‡
Purkinje cell deficient			
Control (3)	55 ± 1	9.2 ± 2.4	515 ± 134
PCD (3)	28 ± 0	13.7 ± 0.5	384 ± 14
Control (2)	58 ± 2	10.8 ± 1.3	626 ± 75
Nervous (2)	36 ± 1	15.5 ± 1.5	558 ± 54
All controls (5)	57 ± 2	9.9 ± 2.1	564 ± 120
All mutants (5)	32 ± 4	14.4 ± 1.4	461 ± 45
Granule cell deficient			
Control (4)	58 ± 3	8.9 ± 3.7	516 ± 215
Weaver (4)	21 ± 8	12.9 ± 0.9	271 ± 19§

Binding experiments were conducted as described.
*Mean ± SD of the number of mice shown in parentheses.
†Mean ± SD. Scatchard analysis was used for each mouse individually, and the B_{max} values were averaged. In all control and mutant mice, the K_d was about 8×10^{-10} M.
‡Product of second and third columns.
§$P < 0.05$, control vs. weaver by two-tailed Student's t test.

Table 2. ET-stimulated InsP_x accumulation in mutant mouse cerebella

Mutant mouse	[^{3}H]Inositol phosphate accumulation, cpm × 10^{-3} Basal*	+ 1 μM ET-3*	ET stimulation, % of basal
Purkinje cell deficient			
Control (3)	5.72 ± 0.87	10.3 ± 0.48	185 ± 36
PCD (3)	8.57 ± 2.15	60.4 ± 27.5	686 ± 182†
Control (2)	4.56 ± 2.06	9.09 ± 2.09	225 ± 56
Nervous (2)	7.57 ± 0.57	45.4 ± 6.48	597 ± 41‡
All controls (5)	5.35 ± 1.57	9.80 ± 1.49	200 ± 49
All mutants (5)	8.17 ± 1.77	54.4 ± 22.9	650 ± 150§
Granule cell deficient			
Control (4)	5.63 ± 1.62	17.3 ± 5.04	324 ± 103
Weaver (4)	16.60 ± 0.22	43.4 ± 1.08	258 ± 45

The accumulation of total [^{3}H]inositol phosphates was determined as described.
*Values represent the mean ± SD for the number of mice shown in parentheses. Experimental data were in triplicate for each mouse. Protein content of tissue per sample was constant for all control and mutant mice and thus was not required for standardization of data.
†$P < 0.005$, control vs. PCD, two-tailed Student's t test.
‡$P < 0.01$, control vs. nervous.
§$P < 0.005$, control vs. all Purkinje cell mutants.

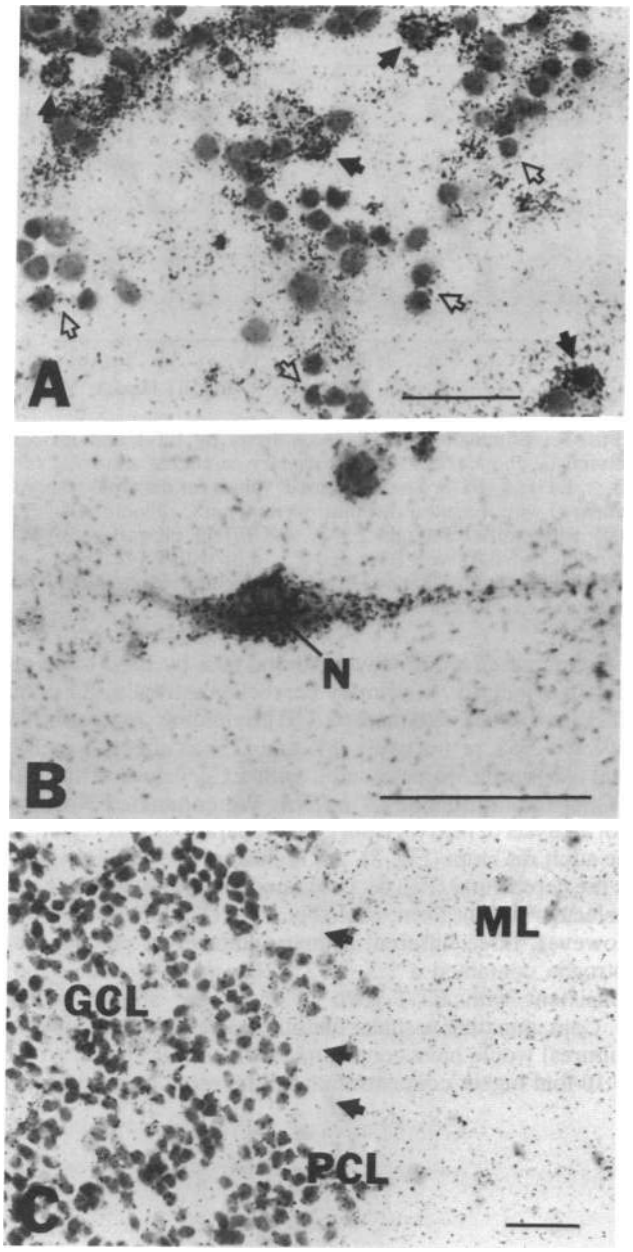

FIG. 1. ET binding sites localized by ^{125}I-ET-1 bound to cultured cells or tissue sections. Addition of 1 μM unlabeled ET-1 reduced the signal level to the background level (<10% of grains; not shown). (*A*) An enriched cultured population of primary early postnatal rat cerebellar granule cells. Cell processes were only lightly stained by hematoxylin/eosin (H&E stain). Filled arrows indicate densely labeled cells; open arrows indicate lightly labeled cells. (*B*) A postnatal rat cerebellar astrocyte in culture stained by H&E stain. (*C*) Adult mouse cerebellum stained lightly with toluidine blue so as not to hide grains. Arrows indicate Purkinje cells. GCL, granule cell layer; ML, molecular layer; PCL, Purkinje cell layer. (Bar = 50 μm.)

devoid of granule cells, ET-stimulated inositolphospholipid turnover was not significantly different from that in control animals. The greater basal inositolphospholipid turnover in mutant mice appeared to reflect their greater incorporation of [^{3}H]inositol into phospholipids (data not shown).

ET Binding Sites in Cultured Cells. The significant decrease in ET receptor binding in mice lacking granule cells but not in mice lacking Purkinje cells suggests an association of ET binding with granule cells. The augmented ET-stimulated inositolphospholipid turnover in PCD and nervous mice and the preservation of ET-stimulated inositolphospholipid turnover in weaver mice suggest an association of ET receptors with nonneuronal cellular elements. Accordingly, we conducted an autoradiographic analysis of ^{125}I-ET-1 binding to cultured neonatal rat cell populations greatly enriched in either granule or astrocytic cells (Fig. 1). Grains located specifically over both cell types demonstrated that both possess ET-1 binding sites. In slices of whole mouse or rat cerebellum, we observed a diffuse pattern of autoradiographic grains over the granule cell and molecular layers with higher densities in the granule cell layer, similar to observations of others in rat cerebellum (9). Unlike whole cerebellum, individual cultured granule cells displayed a heterogeneity in the density of ^{125}I-ET-1 binding sites (Fig. 1).

To characterize the ET binding sites in glia, we examined the binding of ^{125}I-ET-1 to membranes prepared from C_6 glioma cells as well as primary cultures of rat cerebellar astroglia grown on slides (Fig. 2). In control rat cerebellar membranes, ^{125}I-ET-1 bound saturably with a K_d of $\approx 8 \times 10^{-10}$ M and a B_{max} of 10 pmol/mg of protein. ET-3 competed for binding with an IC_{50} of about 0.2 nM, $\approx$4-fold more potent than ET-1. The affinity of ET-1 for astroglial receptors ($K_d = 1 \times 10^{-10}$ M) and glioma receptors ($K_d = 2 \times 10^{-9}$ M) resembled cerebellar membrane binding. ET-1 was more potent than ET-3 in both cell types; however, the similar potencies of ET-1 and ET-3 in primary astroglia resembled cerebellar membranes, while in C_6 glioma cells, ET-3 was only 1% as potent as ET-1 ($K_d = 2 \times 10^{-7}$ M).

ET Stimulation of Inositolphospholipid Turnover in Cultured Cells. ET-1 or ET-3 (1 μM) produced a maximal 2- to 4-fold enhancement of inositolphospholipid turnover in slices of adult rat cerebella (Table 3) as observed previously (5). The EC_{50} values for ET-1 and ET-3 were similar (both 70 nM; Fig. 3), but both were greater than the IC_{50} concentrations for ^{125}I-ET-1 binding probably because of the limited diffusion

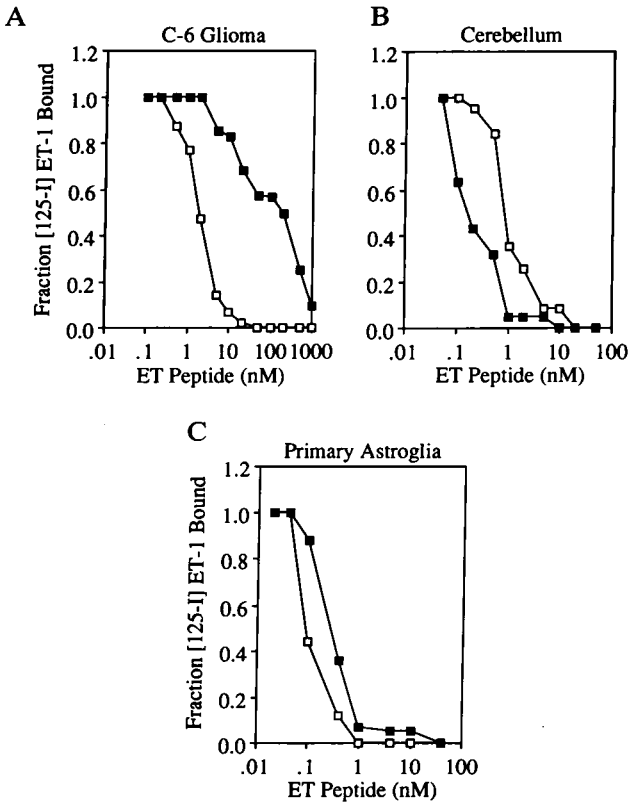

FIG. 2. Concentration-dependent inhibition of ^{125}I-ET-1 binding by ET-1 and by ET-3. Binding assays were as described in text. (*A*) C_6 glioma cells. (*B*) Adult rat cerebellum. (*C*) Primary astroglia. □, ET-1; ■, ET-3.

Table 3. ET-stimulated inositolphospholipid turnover in cultured cells

Cell or tissue	Treatment	InsP_x accumulation,* cpm/μg of protein	% of basal
Adult cerebellum	Basal	6.6 ± 0.6	—
	1 μM ET-3	35 ± 7	530
C_6 glioma cells	Basal	24 ± 3	—
	1 μM ET-3	176 ± 5	720
Primary astroglia	Basal	283 ± 2	—
	5 nM ET-1	716 ± 1	250
	5 nM ET-3	608 ± 5	210
	1 μM ET-3	683 ± 80	240
Primary granule cells	Basal	356 ± 28	—
	1 μM ET-3	627 ± 91	180

*The accumulation of total [^{3}H]inositol phosphates was determined as described and corrected for total protein per assay. Values represent the mean ± SD for triplicate determinations from a representative experiment. The experiment was replicated twice.

through tissue slices. Basal inositolphospholipid turnover in C_6 glioma cells was 4-fold greater than in cerebellar slices when correction was made for protein. ET-1 or ET-3 (1 μM) increased inositolphospholipid turnover 2- to 6-fold in C_6 glioma cells (Table 3 and Fig. 3). The EC_{50} concentrations for ET-1 and ET-3, 1 nM and 200 nM, respectively, also correlated with affinities for ET binding sites (Fig. 3). In primary astroglia, ET-3 increased inositolphospholipid turnover about 150%. Basal inositolphospholipid turnover was 40-fold greater than in cerebellar slices when corrected for total protein. The similar potency of ET-1 and ET-3 (5 nM for maximal response) is more like cerebellar than C_6 glioma receptor responses. In C_6 glioma cells and primary astroglia, inositolphospholipid turnover enhancement was absolutely dependent upon calcium and was eliminated by 1 mM EGTA (data not shown).

ET-3 increased inositolphospholipid turnover 80% in primary cultures of rat cerebellar granule cells with basal levels similar to those of primary astroglia (Table 3).

ET Enhancement of Glial Mitogenesis. ET potently stimulated glial cell proliferation (Fig. 4). In C_6 glioma, ET-1 (EC_{50}, 0.05 nM) was markedly more potent than ET-3 (EC_{50}, 100 nM) in enhancing [^{3}H]thymidine incorporation, but ET-3 was more efficacious. The suboptimal effect of ET-1 at higher doses was reproducible in three separate experiments. ET-1 and ET-3 were 7% and 24% as effective as 10% fetal calf serum, respectively. Within 24 hr, cell numbers were maximally increased by 20% by ET-1 and 60% by ET-3 (data not shown). Similarly, in primary cerebellar astroglia, ET-1 and ET-3 maximally augmented [^{3}H]thymidine incorporation 15% and 23%, respectively (8% and 11% as effective as 10% fetal calf serum, respectively), with EC_{50} values of 0.1 nM.

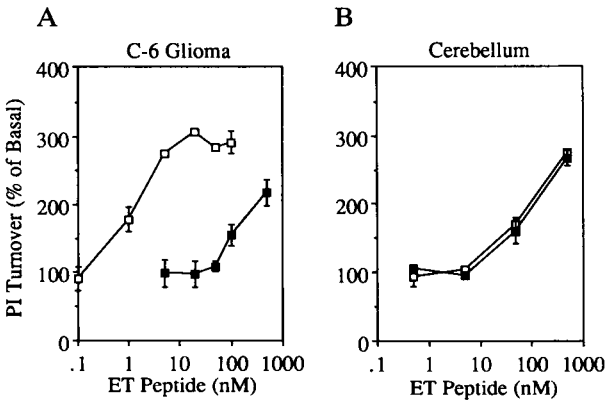

FIG. 3. Concentration dependence of ET-stimulated inositolphospholipid turnover measured as described. Values are the ratios of the means ± SD for triplicate determinations of basal and stimulated inositolphospholipid turnover repeated twice. (*A*) C_6 glioma cells. (*B*) Adult rat cerebellum. □, ET-1; ■, ET-3.

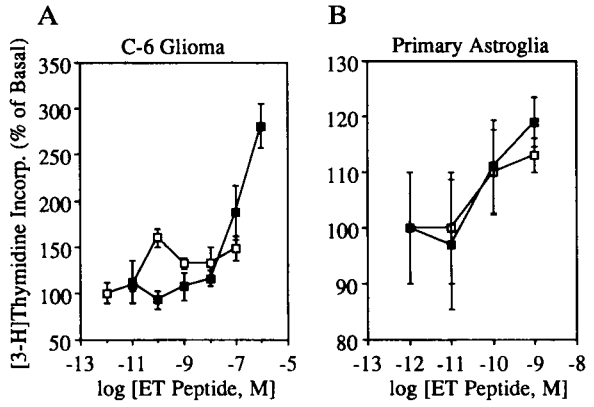

FIG. 4. Stimulation of DNA synthesis by ET-1 and ET-3 in cultured C_6 glioma cells (*A*) and primary cerebellar astroglial cells (*B*). □, ET-1; ■, ET-3. Student *t*-test *P* values for maximal response compared with the basal response were: (*i*) in C_6 glioma cells, $P < 0.005$ with both ET-1 and ET-3, and (*ii*) in primary cerebellar astroglia, $P < 0.05$ with ET-1 and $P < 0.01$ with ET-3. Values are from triplicate determinations from a representative experiment of at least three experiments with similar results.

Glial Localization of ET mRNA. We conducted Northern blot analysis of mRNA from glial preparations and regions of the adult rat brain (Fig. 5). All regions of the rat brain, such as the cerebellum (Fig. 5), contained predominantly a higher molecular weight form, probably ET-3 (3) (data not shown). However, three different primary cultures of postnatal rat astroglia contained a 2.3- to 2.5-kDa mRNA species most consistent with ET-1 (two of these are shown in Fig. 5). Contaminating vascular endothelial cells (<0.1% of some cultures) would have contributed little to this signal. At a 5- to 10-fold higher concentration on the same membranes, C_6

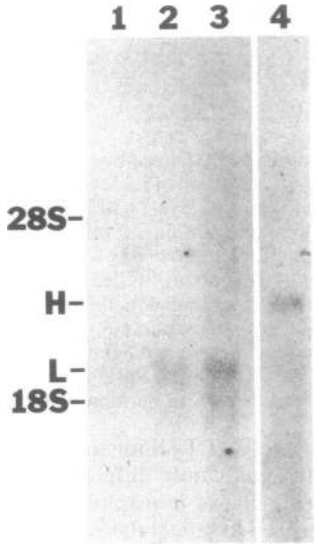

FIG. 5. Northern blot analysis of total RNA from glial preparations and adult rat cerebellum labeled with an ET-specific probe. Bands 18S and 28S were used as size markers. H, higher molecular weight form; L, lower molecular weight form. Lanes: 1, C_6 glioma cells (≈10 times more RNA loaded than in lanes 2 or 3); 2, postnatal rat brain astroglial cells; 3, postnatal rat cerebellar astroglial cells; 4, adult rat cerebellum.

glioma mRNA failed to reveal any mRNA species homologous to our ET probe (Fig. 5).

DISCUSSION

Our findings indicate both glial and neuronal roles for ET in the brain. Evidence for ET effects in glia include: *in vivo*, ET-stimulated inositolphospholipid turnover is greater or unchanged in mutant mice lacking Purkinje cells or granule cells when compared with control mice. The enhancement of ET-stimulated inositolphospholipid turnover in the mutant mice may reflect increased numbers of astroglial cells as neurons are lost (18). *In vitro*, ET receptor binding sites, ET-stimulated inositolphospholipid turnover, and ET promotion of mitogenesis are demonstrable in two preparations of glial cells.

A neuronal, granule cell locus for ET actions is also suggested by the decline in ET binding in granule cell-deficient mice and the presence of ET binding sites on primary cultured granule cells. Moreover, we observed ET stimulation of inositolphospholipid turnover in these cultures of granule cells, confirming findings of Lin *et al.* (20). ET-elicited inositolphospholipid turnover in granuloprival cerebella resembled control animals and was less than in Purkinje cell-deficient animals. Thus, the granule cell-deficient mutants may have lost the granule cell component of inositolphospholipid turnover while augmenting the glial component. The Purkinje cell-deficient mutants presumably preserved the granule cell component and increased the glial component.

The potencies of ET-1 and ET-3 in competing for ^{125}I-ET-1 binding, stimulating inositolphospholipid turnover, and promoting mitogenesis differ markedly in C_6 glioma cells and primary cerebellar astroglia, which might reflect subtypes of ET receptors (21, 22). The absolute and relative potencies of ET-1 and ET-3 in augmenting glial cell DNA synthesis resemble their potencies in stimulating inositolphospholipid turnover and in competing at ET receptor binding sites. Thus, the same or closely similar receptors appear involved in ligand binding, inositolphospholipid turnover, and mitogenesis. Supattapone *et al.* (23) recently observed an ET-induced rise in intracellular calcium in astroglia in calcium-free medium, presumably secondary to IP_3 release, as well as ET-enhanced astroglial mitogenesis similar to results presented here. Whether ET-stimulated inositolphospholipid turnover is causally associated with proliferation in glial cells is not certain; however, inositolphospholipid turnover has been implicated in growth in numerous cell types (24) including glia (25).

The cellular source of ET in the brain has not been clarified. The ET mRNA we observe in rat glial preparations (probably ET-1) differs from the predominant form of adult rat brain (probably ET-3). We suspect that ET-3 normally present in brain is synthesized in neurons. Glial ET-1 mRNA might be synthesized by astrocytes only during development or active gliosis and not in normal adult brain. ET receptor binding sites on glia also appear to have higher affinity for ET-1 than ET-3.

The biosynthesis of ET within glia and its actions upon glia may reflect an autocrine or paracrine type of action. Similarly, in peripheral tissues ET is formed in endothelial cells and acts on closely adjacent smooth muscle cells (3, 4). In smooth muscle as in glia, ET promotes inositolphospholipid turnover and mitogenesis (6). The vasoconstrictor and cell growth actions of ET initiated by substances such as thrombin and transforming growth factor β may reflect responses to tissue injury. In the brain, ET enhancement of inositolphospholipid turnover and mitogenesis in astroglia may also mediate responses to neuronal injury. Certain other peptide receptors, such as those for substance P (26), are only expressed on glia after neuronal injury. Conversely, ET synthesized in glia at sites of injury could promote wound healing responses in glial or other cells. Fuxe *et al.* (27) have demonstrated immunoreactive ET in reactive astrocytes in the hippocampal formation of rats with ibotenic acid-elicited neuronal destruction. Thus, ET may be one of several peptide mediators involved in the glial response to injury in the brain.

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