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Columnar activity regulates astrocytic β -adrenergic receptor-like immunoreactivity in V1 of adult monkeys

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Abstract

Recent results indicate that astrocytic β -adrenergic receptors (β AR) participate in noradrenergic modulation of synaptic activity. In this study, we sought to examine whether neural activity can, in turn, regulate astrocytic β AR. To address this question, an antiserum that recognizes β -adrenergic receptors (β AR) specifically in astrocytes was used to assess the distribution of the receptors across ocular dominance columns in V1 of two monocular and four visually intact adult monkeys. Cytochrome oxidase histochemistry (CO) was used to identify the position of the cortical laminae and of the ocular dominance columns receiving visual inputs from the intact and enucleated eyes. This stain revealed the expected pattern within V1 of monocular monkeys –i.e. darker and lighter bands of equal widths (*ca.* 500 μ m) spanning laminae 4–6, each associated with larger and smaller blobs, respectively, in lamina 2/3. Alignment of CO sections with adjacent sections stained for astrocytic β AR by the immunoperoxidase method revealed intense β AR-like immunoreactivity (β AR-li) in the superficial laminae, a slightly weaker staining in the infragranular laminae and weakest staining in lamina 4C. Within lamina 4C, a prominent striped pattern was evident. The darker bands of the stripe closely matched widths and positions of the lighter CO columns associated with the enucleated eye. On the other hand, immunocytochemical staining for the astrocytic intermediate filament protein, GFAP, within V1 of monocular monkeys revealed no inter-columnar difference in the density of astrocytic cell bodies or processes. Nissl stain also revealed no overt inter-columnar differences in cell density. V1 of visually intact monkeys exhibited a similar laminar distribution pattern of β AR-li and of CO. Within lamina 4C, β AR-li was uniformly faint and CO staining was uniformly intense. This suggests that the striped pattern of β AR-li seen in lamina 4C of monocular monkeys results from elevation of the β AR-antigen within the inactive columns. The results indicate that astrocytic β AR density is regulated by local neural activity. The mechanisms regulating β AR density are likely to be independent of those regulating glial cell proliferation or GFAP synthesis. *In vitro* experimental results of others suggest that elevation of astrocytic β AR may be a mechanism compensating for chronic neural inactivity, since the coincident release of noradrenaline with visual stimulation would elevate neuropil excitability *via* the astrocytic mechanism of (1) decreasing the uptake of neuronally released L-glutamate; (2) increasing GABA uptake; and (3) stimulating glycogenolysis. Alternatively, the changes in β AR-li may reflect an up-regulation of the receptors within inactive columns due to reduced levels of noradrenaline release.

Keywords

Astrocytes; Catecholamine receptors; Activity-dependent regulation; Monocular deprivation; V1

Introduction

Recent advances in the localization of neurotransmitters, receptors, and receptor-linked proteins have increased our understanding of chemical transmission within the central nervous system (CNS). It has been shown that receptors as well as other receptor-linked proteins can occur extrajunctionally in neurons (Aronin & DiFiglia, 1992; Aoki et al., 1992) as well as in glia (reviewed in Fedoroff & Vernadakis, 1986). Moreover, serial electron microscopy has confirmed that not all axonal varicosities are associated with morphologically identifiable synaptic junctions (Séguéla et al., 1990; Descarries et al., 1991; Palay, 1991) and when they are, astrocytic processes often are juxtaposed to the synapses (Peters et al., 1991). These observations indicate that chemical transmission may involve astrocytes and extrajunctional sites on neurons in addition to morphologically identifiable synapses.

As for the central noradrenergic system, *in vitro* and *in vivo* studies have shown that activation of astrocytic β -adrenergic receptors (β AR) can cause extension of astrocytic processes (Narumi et al., 1978; Bicknell et al., 1989; Hatton et al., 1991). These processes can interdigitate between terminals and their targets, thereby limiting chemical transmission. Alternatively, the extended processes can surround synaptic junctions to isolate them from the surrounding neuropil (Tweedle & Hatton, 1980; Hatton et al., 1984, 1991; Aoki & Pickel, 1992a,b). Activation of astrocytic β AR also increases astrocytes' uptake of GABA and decreases astrocytic uptake of L-glutamate (Hansson & Ronnback, 1992) while also stimulating glycogenolysis (Quach et al., 1988; review by Stone & Ariano, 1989). Noradrenaline is reported to modulate synaptic strength and to increase the ratio of stimulus-driven activity relative to spontaneous activity (Neuman & Harley, 1983; Hopkins & Johnston, 1984; Stanton & Sarvey, 1985; reviewed by Foote & Morrison, 1987). These noradrenergic actions may depend on the above-described mechanisms involving astrocytic β AR in addition to the well-characterized presynaptic and postsynaptic mechanisms (McCormick et al., 1991; Harley, 1991; Segal et al., 1991). The aim of the current study was to examine whether the density of astrocytic β AR may, in turn, be regulated by synaptic activity and, in this way, mediate long-term alterations in synaptic strengths.

The visual cortex is a useful model for studying activity-dependent regulation of molecules. This is because (1) afferents arising from the two eyes form spatially segregated, yet, interdigitated domains in lamina 4C, i.e. the ocular dominance columns; (2) monocular stimulation is easily achieved; and (3) while GABA and tachykinin immunoreactivity within interneurons may be reduced (Hendry & Jones, 1986 & 1988; Hendry et al., 1988), the majority of postsynaptic neurons in the main thalamo-recipient zone, lamina 4C, do not degenerate following the administration of monocular deprivation or enucleation in adulthood (Hendry & Jones, 1986, 1988). This last observation probably indicates that activity arising from non-thalamic inputs continue to drive the "deprived" layer 4C cells sufficiently to prevent degeneration. However, relatively little is known about activity-dependent regulation of astrocytes in this model. Thus, we sought to determine whether astrocytic β AR exhibit intercolumnar differences in the visual cortex of monocular monkeys. For the detection of β AR on astrocytes, we used an antibody directed against the C-terminal tail portion of the receptor molecule (amino acids 404–418) which was previously shown to recognize β AR or β AR-like antigens in astrocytes, and not neurons, in the adult rat and cat visual cortices (Aoki, 1992).

Materials and methods

Source of histological reagents

The rabbit antiserum recognizing a synthetic polypeptide corresponding to residues 404–418 at the C-terminal tail of hamster lung β -adrenergic receptor (β_2 -subtype), was a generous gift

from Dr. C.D. Strader of Merck, Sharp, and Dohme Research Labs. (Rahway, NJ). This antibody recognizes a single protein species of predictable size by the Western blot method (Strader et al., 1987a) and immunoprecipitates a protein species with β_2 AR's ligand-binding properties (Strader et al., 1987b). Previous immunocytochemical studies demonstrated that the antiserum immunolabels astrocytes, and not neurons, within intact adult rat and cat CNS (Aoki & Pickel, 1992a,b; Aoki, 1992). Biotinylated goat anti-rabbit IgG and avidin-biotin-peroxidase (ABC) kit, used for visualization of the antibody-antigen complex, were obtained from Vector Labs., CA. Anti-glial fibrillary acidic protein (GFAP), used to immunolabel astrocytes, was purchased from Sigma Chemical Co., St. Louis, MO. Cytochrome oxidase, cytochrome c and catalase, used for the identification of ocular dominance columns, also were purchased from Sigma. 3,3'-diaminobenzidine tetrahydrochloride was purchased from Aldrich Chemical, Milwaukee, WI.

Preparation of visual cortical sections

Brains of two male monocular monkeys (*Macaca mulatta*), approximately 3 years of age, were generously donated to us by Dr. D. Kleinberg at the NY Veterans' Affairs Lab. At the age of 8–12 months, these animals had been castrated; at 2 years of age, they were hypophysectomized and unilaterally enucleated, then given growth hormone treatments for a 1-year period for the purposes of Dr. Kleinberg's study. Aldehyde-fixed brains were prepared from these monkeys approximately 1 year following enucleation (at the age of 3 yrs.). Monkeys were deeply anesthetized using ketamine (10 mg/kg, i.m.) in combination with sodium pentobarbital (50 mg/kg, i.v.) by the veterinary staff at New York University. They were then transcardially perfused at a flow rate of 70 ml/min with 100 ml of heparinized phosphate-buffered saline (PBS) followed by 2 l of aldehyde fixatives buffered to pH 7.4 using 0.1 M phosphate buffer (PB). The aldehyde fixative was 2 l of 4% paraformaldehyde for one animal. The other received a 250-ml bolus of 3.75% acrolein (King et al., 1983) and 2% paraformaldehyde, followed by perfusion with 1.75 l of 2% paraformaldehyde. The brains were immediately removed from the skull, then stored in 2% paraformaldehyde overnight at 4°C.

Aldehyde-fixed brain hemispheres of four visually and hormonally intact monkeys (*Macaca fascicularis*) were generously donated to us by members of the Department of Neurology at Cornell University, Medical College, NY, and the Center for Neural Science at New York University. Paraformaldehyde- and acrolein-fixed tissue were obtained from these monkeys using identical procedures of intracardial perfusions following the administration of 2-ml Surital or 50 mg/kg sodium pentobarbital i.v. at the end of the donors' neurophysiological experiments.

Tangential and parasagittal sections spanning area V1 of the cerebral cortex were prepared from these brains using a vibratome or a freezing microtome at a setting of 40 μ m. All sections were collected in 0.1 M PB. Sections obtained from acrolein-fixed brains were immersed in 1% sodium borohydride/PB solution for 30 min to terminate aldehyde-fixation (King et al., 1983), then rinsed repeatedly in 0.1 M PB until bubbles ceased to emerge from sections. These sections were transferred to saline solution buffered with Tris at pH 7.4 (TBS).

Immunocytochemical procedure

Free-floating sections designated for β AR immunocytochemistry were incubated overnight at room temperature in a dilution of rabbit anti- β AR antiserum. The antiserum was diluted 1:1000 using a buffer consisting of 0.1 M Tris-buffered saline (TBS) and 1% BSA both in the presence and absence of 0.3% Triton X-100. Sections designated for GFAP immunocytochemistry were incubated at room temperature in a rabbit anti-GFAP antiserum diluted 1:1000 using TBS-BSA in the presence of 0.3% Triton X-100. Sections designated for controls were incubated in the absence of GFAP or β AR antiserum. On the following day, these sections were rinsed

in TBS, then incubated in a 1:200 dilution of biotinylated goat anti-rabbit IgG. The antigen-antibody complex was visualized by the avidin-biotin-HRP complex method (Hsu et al., 1981). Sections were mounted on gelatin-coated slides, coverslipped, then viewed or photographed under a Zeiss Axiophot light microscope using bright-field and differential interference contrast optics.

Camera lucida drawing

The GFAP-immunoreactive processes and cell bodies within lamina 4C of V1 of monocular monkeys were mapped to ascertain the relationship between GFAP immunoreactivity and β AR-like immunoreactivity (β AR-li) within astrocytes. The sections stained for GFAP immunoreactivity were immediately adjacent to those stained for CO and were two sections removed from those stained for β AR. The GFAP-stained sections were viewed using a 20 $\times$ objective and drawn at a final magnification of 190 $\times$. The drawn images were reduced in size using a photocopier to match the magnification of micrographs of CO-stained sections printed at a final magnification of 39 $\times$. The CO micrographs and camera lucida drawings were matched using blood vessels as fiduciary marks.

Cytochrome oxidase (CO) histochemistry

Sections immediately adjacent to β AR-immunolabeled sections were processed for CO histochemistry using previously described methods (Wong-Riley, 1979). CO histochemistry allowed for visualization of ocular dominance columns. The sections were mounted on gelatin-coated microscope slides, then viewed or photographed under bright-field optics using the Zeiss Axiophot light microscope.

Results

Results obtained from cytochrome oxidase histochemistry and β AR immunocytochemistry were independent of the fixation condition. Thus, results obtained from the two conditions will be described together.

V1 of visually intact monkeys

Sections permeabilized using Triton X-100 showed a distinctly laminar distribution of β AR-like immunoreactivity (β AR-li). Comparison of these sections with the immediately adjacent sections, stained for cytochrome oxidase (CO) to identify laminar positions, revealed the lamina exhibiting the least staining to be 4C (Figs. 1A and B). The most intense β AR labeling occurred in the superficial laminae 1, 2, and 3, followed by lamina 5 and the deepest portion of lamina 6. The staining consisted, in part, of roughly circular puncta, 50–100 μ m in diameter. Examination of these profiles at a higher magnification revealed these puncta to be clusters of fine processes, some of which appeared interwoven (Fig. 1C), others of which radiated from a point (Fig. 1D). These resembled astrocytic processes previously visualized by the Golgi stain (reviewed by Vaughn, 1984). β AR-li was also evident within fibrous astrocytes forming the glial limitans in lamina 1 and distributed throughout the white matter (not shown but see the paragraph below).

Sections immunolabeled for β AR in the absence of Triton X-100 exhibited a laminar distribution pattern similar to that seen in the presence of Triton X-100 (not shown). Examination of these sections at a higher magnification, however, revealed two major differences: (1) diminished labeling of astrocytic processes in laminae 2–5; and (2) a more prominent labeling of small (10–20 μ m diameter) neuronal cell bodies throughout the laminae (Fig. 1G). On the other hand, immunoreactivity within fibrous astrocytes in lamina 1 and in the white matter were unaffected by the removal of Triton X-100 (Figs. 1E and F).

Results from controls

Sections processed for immunocytochemistry but in the absence of the β AR antiserum showed only diffuse, light staining that did not vary across ocular dominance columns or laminae (not shown).

V1 of monocular monkeys

Cytochrome oxidase histochemistry (CO) revealed the expected staining pattern (Hevner & Wong-Riley, 1990) within tangential sections spanning cortical area V1 of both monocular monkeys (Fig. 2A). Layers 2/3 were easily identified by the presence of larger and smaller blobs representing neuropil driven by the intact and enucleated eyes, respectively. A narrow band with a “honeycomb” pattern, previously identified to correspond to lamina 4A (Hevner & Wong-Riley, 1990), also was evident. The region beyond lamina 4A exhibited a markedly striped staining pattern, followed by a more subtly striped region. Again, comparison with previous results (Hevner & Wong-Riley, 1990) revealed these to correspond to laminae 4C and 5/6, respectively. Within these laminae, the darkly stained bands presumably belonged to the active columns while the light bands corresponded to the inactive columns. Finally, a few pyramidally shaped large perikarya were evident in the deepest portion of the cortical grey matter corresponding to lamina 6. The blood vessel lumens were clearly evident as unstained profiles within heavily stained portions of the sections (small arrows in Fig. 2A).

Since our interest was to study the distribution of *astrocytic* β AR, all sections obtained from monocular monkeys were incubated in the β AR antiserum in the presence of Triton X-100 to optimize astrocytic labeling. Macroscopically, all β AR-immunolabeled sections collected from the paraformaldehyde- and acrolein-fixed brain exhibited laminar and columnar patterns (Figs. 2B and 4A). Alignment of these with adjacent sections stained for CO revealed that the staining is most intense in the supragranular laminae, followed by lamina 5 and the deepest portion of lamina 6 and least intense in lamina 4C. This was as seen for V1 tissue of visually intact monkeys. In addition, lamina 4C exhibited darker bands that ran in the direction of ocular dominance columns. Alignment of CO and β AR sections using blood vessels as fiduciary marks revealed that widths and positions of the dark β AR bands consistently match with the inactive ocular dominance columns. The columnar heterogeneity in staining was relatively more subtle in the infragranular laminae when compared to lamina 4C and not apparent in the supragranular laminae.

As was seen in V1 of visually intact monkeys, much of the β AR-li consisted of punctate profiles ranging from 50–100 μ m in diameter. Examination of these profiles at a higher magnification confirmed these to be a meshwork of fine processes (Fig. 2B inset), as was described above for V1 of normal monkeys.

To ascertain whether the striped distribution of β AR-li in lamina 4C reflected intercolumnar differences in the densities of astrocytes, immunocytochemistry was performed using an antiserum directed against the astrocytic marker, GFAP. The distribution of GFAP-immunoreactive processes was heterogeneous. To determine whether the heterogeneity followed the CO pattern of staining, the position of all identifiably GFAP-immunoreactive processes within randomly chosen sectors of lamina 4 were recorded using the camera lucida. Alignment of these drawings with micrographs taken from sections stained for CO revealed that these heterogeneities do not follow boundaries of ocular dominance columns (Figs. 3 and 4B).

Discussion

We have shown a strikingly laminar distribution of astrocytic β AR-li in V1 of normal and monocular monkeys. Comparisons of CO-stained sections with β AR-stained sections obtained from brains of monocular monkeys revealed astrocytic β AR-li within lamina 4C to be greater in the inactive ocular dominance columns than in the active columns. However, GFAP immunoreactivity within lamina 4C of monocular monkeys shows no intercolumnar differences in staining. Therefore, the cellular mechanisms regulating astrocytic GFAP immunoreactivity must be distinct from those regulating astrocytic β AR-li. Moreover, since β AR-li is uniformly sparse within lamina 4C of visually intact monkeys, the striped pattern of β AR-li within lamina 4C is likely to result from an increase in β AR-like antigen within the inactive columns. Discussed below are considerations regarding (1) the specificity of this effect, (2) the molecular mechanisms that may link neural activity to astrocytic β AR-li, and (3) possible functional significance of the altered levels of astrocytic β AR-li.

Specificity of β AR-li for astrocytes

Although the antiserum used in the present study did not recognize neurons within tissue permeabilized with Triton-X 100, we do not interpret this result to reflect an absence of neuronal β AR. Previous studies demonstrated that an antibody directed against the third intracellular loop portion of the receptor recognizes neurons exclusively (Aoki et al., 1989), while the antibody used in the present study recognizes astrocytes and no neurons (Aoki & Pickel, 1992a,b; Aoki, 1992). Thus, the observed cellular distribution of immunoreactivity was as expected. Since neurons and astrocytes both express β AR (Burgess & McCarthy, 1985; Salm & McCarthy, 1992; Sutin & Shao, 1992), we hypothesized earlier (Aoki & Pickel, 1992a; Aoki, 1992) that the differential distribution of immunoreactivity to the two anti-sera might reflect differences in the conformational state of the molecule, rather than absence or presence of the molecule. At this time, we have no additional evidence that proves or disproves this hypothesis. Thus, it is possible that the intercolumnar differences in β AR-li within astrocytes reflect altered conformational states of the molecule rather than altered concentrations of the protein species within astrocytes. Whichever is the case, the antibody has been shown to recognize a single protein species with β AR's ligand-binding properties (Strader et al., 1987b). Thus, immunoreactivity within tissue also is likely to reflect ligand-binding sites.

The antigen used for the production of the antiserum was a synthetic polypeptide whose amino acid sequence corresponds to residues 404 through 418 of hamster lung β AR (β_2 -subtype) (Strader et al., 1987a). To date, the amino acid sequence of this antigen remains unique among the G-protein linked receptor molecules cloned. The antiserum recognizes a single band of protein by the Western blot method (Strader et al., 1987a) and immunoreactivity of COS cells transfected with the β AR gene is abolished when the antiserum is preadsorbed with the antigenic peptide (Strader et al., 1987b). Within tissue, no immunoreactivity is detectable when the antiserum is eliminated from the incubation medium. Although these observations are supportive of the specificity of the immunocytochemical procedure, the possibility that the antiserum cross-reacts with a non- β AR molecule cannot be excluded entirely. For these reasons, we have maintained the use of the term, *β AR-like*, to remind readers and ourselves of the possibilities of cross-reactions of the primary antiserum.

Alterations in astrocytic β AR-li may reflect alterations in the concentration or conformation of the receptor but not in astrocytic density

Since the enucleation procedure involves severing of retinal ganglion cell axons, transsynaptic anterograde degeneration was expected to occur in the lateral geniculate nucleus (LGN). Indeed, Nissl staining of this structure revealed decreases in the density of cell bodies and blood vessels within layers 1, 4, and 6 of the LGN contralateral to the enucleated eye and within

layers 2, 3, and 5 of the ipsilateral LGN (not shown). However, Nissl staining of the visual cortex revealed no overt columnar differences in cell density and CO histochemistry revealed no change in the *width* of active *vs.* inactive ocular dominance columns. This was as reported previously for V1 of animals enucleated or visually deprived in adulthood (Hevner & Wong-Riley, 1990; Hendry & Jones, 1986, 1988). Presumably, monkeys of this study were adult-like from the point of view of V1 cytoarchitecture when they were enucleated at the age of 1 year. Camera lucida drawings of GFAP staining revealed no inter-columnar differences in GFAP staining. This observation suggests that the columns representing the enucleated eye exhibit no apparent gliosis 1-year post-operation, even if enucleation may have caused transient, local gliosis within the inactive columns. Thus, the inter-columnar differences in astrocytic β AR, seen at 1-year post-enucleation, cannot be explained simply by the proliferation of astrocytes or expansion of astrocytic processes.

Although it is possible that a quantitative assessment of GFAP distribution is necessary for detecting gliosis, there are at least two other interpretations of the observed difference between the astrocytic β AR- and GFAP-immunolabeling pattern. One is that increased levels of β AR-li reflect alteration in a population of cells not expressing GFAP – these may be non-astrocytic or they may be immature astrocytes that have yet begun expressing GFAP. We do not favor this hypothesis, since there is no reason to speculate the existence of immature astrocytes within V1 1 year after enucleation. Moreover, the population is probably not one of oligodendrocytes or microglia, since electron microscopy, which is a facile method for identifying these types of cells, revealed no detectable immunoreactivity in their cell bodies or processes (Aoki & Pickel, 1992a,b; Aoki 1992). We cannot exclude the possibility that they are neurogliaform cells, speculated to be GABAergic neurons (Jones, 1984), although the number of processes radiating from the cell bodies appeared more numerous than is characteristic for such cells.

Another interpretation derived from comparing the macroscopic distribution of β AR-li to GFAP immunoreactivity is that astrocytes can regulate receptor densities within their processes independent of the synthesis and intracellular trafficking of GFAP. Studies of individual astrocytes' morphology show that, in cells where β AR-li is detectable, the β AR stain resembles more that of Golgi-stained astrocytes than that of GFAP-stained processes. Presumably, the reason for the striking differences in astrocytic morphology as seen by the Golgi method *vs.* GFAP immunocytochemistry is that almost all of the processes can be silver-impregnated by the Golgi method, while only a portion of these processes would contain GFAP (Vaughn, 1984). Taken together, these observations indicate that the distribution of β AR within individual astrocytes may be more uniform than that of GFAP.

Molecular mechanisms that may link neural activity to β AR density

Astrocytes, grown *in vitro*, express various types of receptors (Hansson, 1988, 1990; Fedoroff & Vernadakis, 1986), including the non-NMDA type glutamate receptors (Cornell-Bell et al., 1990; Barres et al., 1990) and GABA receptors (Kettenmann et al., 1987; MacVicar et al., 1989) besides catecholaminergic receptors and uptake sites (Kimmelberg, 1986). Thus, astrocytes may detect neural activity by chemical transmission involving the binding of ligands to neurotransmitter receptors on the surfaces of astrocytes. Since thalamo-cortical afferents that are enriched in lamina 4C are glutamatergic (Tsumoto, 1990), astrocytes residing in lamina 4C that express glutamatergic receptors would be able to register incoming afferent activity directly. Alternatively, since GABA immunoreactivity is altered by monocular deprivation (Hendry & Jones, 1986, 1988), reduction in GABAergic transmission within inactive columns may be detected by astrocytes bearing GABAergic receptors. Furthermore, astrocytes may register neural activity *via* alterations in extracellular concentration of K^+ (Kuffler & Nicholls, 1966). Any or all of these events may be linked to cellular mechanisms regulating astrocytic β AR density. Finally, alterations in neural activity may reduce the release of noradrenaline

within the local area, thereby evoking supersensitivity of the local tissue *via* increases in functional β AR. In support of this view, noradrenaline release within the cerebral cortex has been documented to be regulated by GABA (review by Chesselet, 1984) and reductions of excitatory inputs, caused by deafferentation, have been shown to result in up-regulation of glial β AR, albeit in conjunction with gliosis 7–28 days post-lesion (Sutin & Shao, 1992).

Functional significance of the altered levels of astrocytic β AR-li

The functional significance of elevation in astrocytic β AR within the inactive columns might be to enhance neuropil excitability (Hansson & Ronnback, 1992). *In vitro* results have shown that activation of β AR elevates GABA uptake: this may limit the binding of GABA onto postsynaptic sites, thereby limiting GABAergic inhibition. *In vitro* results have also shown that activation of β AR decreases L-glutamate uptake, thereby increasing the extracellular concentration of L-glutamate and increasing the duration and/or strength of glutamatergic neurotransmission. Furthermore, glycogenolysis that is stimulated by the activation of astrocytic β AR (reviewed by Stone & Ariano, 1989) may provide an alternative energy source in a zone clearly depleted of mitochondria, as evidenced by the low levels of histochemical reactivity for CO. Finally, elevation of astrocytic β AR may be triggered by the reduced levels of noradrenaline release within the inactive columns: the consequence of this response would be that the neuropil's sensitivity to noradrenaline is enhanced, thereby compensating for reduction in noradrenaline release. Almost all of these results are based on results obtained from *in vitro* experiments: we cannot be sure that the same cellular events operate *in vivo*. If they do, then these mechanisms may serve to compensate for the low levels of incoming activity within the deprived columns by increasing the probability that an afferent volley that is coincident with noradrenaline release leads to excitation of neurons residing in lamina 4C.

In conclusion, we have demonstrated an activity-dependent regulation of glial receptor immunoreactivity within V1 of adult monkeys. In light of this finding, it would be interesting to examine whether noradrenergic innervation or the density of other catecholaminergic receptors exhibit intercolumnar differences following monocular deprivation.

Acknowledgments

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Fig. 1.

Laminar and cellular localization of β AR-li in V1 of visually intact monkeys. Panel A shows a section stained by CO histochemistry. All others show β AR-li. Sections shown in panels B–D were permeabilized by Triton X-100 while the remaining sections were stained in the absence of Triton X-100. Scale bar = 3200 μ m in A and B, 40 μ m in D, and 20 μ m in other panels. A: CO histochemistry allowed for the identification of the laminar positions, marked to the right. B: Comparison of this with an immediately adjacent section stained for β AR reveals prominent bands of labeling in lamina 5 and at the lamina 6/white matter boundary and the least labeling in lamina 4C. Immunolabeling in the superficial laminae appears diffuse. Arrowheads point to immunoreactive puncta that are distributed throughout the laminae. C: Examination of the

immunoreactive puncta here, at a higher magnification, reveals that these consist of a clustered meshwork of processes (arrowhead). D: Here, the puncta consist of a collection of radiating processes of varying calibres (small arrows). E, F: Fibrous processes of astrocytes (small arrows) also are immunoreactive. Arrowheads point to a small immunoreactive cell body in E and to apparent origins of the radiating processes in F. Although these examples were taken from a tissue in which Triton X-100 permeabilization was omitted, similar labeled processes were seen in the presence of Triton X-100. G: Examples of β AR-li profiles found only in the absence of Triton X-100. Arrowheads point to unlabeled nuclei of cell bodies in lamina 4 containing intense immunoreactivity in the perikarya. Small arrows point to small clusters of punctate labeling that are barely resolvable.

Fig. 2.

Comparison of the histochemical stain for cytochrome oxidase (panel A) with the immunocytochemical stain for β AR (panel B) in V1 of a monocular monkey's brain fixed with paraformaldehyde reveals greater intensity of β AR-li within the pale CO columns. Numbers refer to cortical laminae. Scale bar = 1 mm in large panels, 40 μ m in the inset. A: Cytochrome oxidase histochemistry shows a discrete banding pattern that is most evident in the lower half of lamina 4C but persistent through laminae 5 and 6. According to Hevner and Wong-Riley (1990), the darker bands in lamina 4C are of the intact eye's ocular dominance columns, while the lighter bands in the same layer (large arrows) are of the enucleated eye. The "honeycomb" pattern of staining (identifiable in the sections but not visible in this figure) demarks the position

of lamina 4A. Within laminae 2 and 3, larger and smaller “blobs” corresponding to supragranular portions of active and inactive columns interdigitate. B: β AR-li is intense in the superficial and infragranular laminae. A striped pattern is evident within the lower half of lamina 4C. Alignment of this section with the adjacent CO-stained section was achieved using blood vessels as fiduciary marks (small arrows point to their positions). This procedure reveals that the darker β AR-immunoreactive bands (large arrows) match in position and width with the paler CO columns (large arrows in A). Arrowheads point to immunoreactive puncta that occur throughout the laminae, including paler β AR columns in lamina 4C. Examination of the puncta at a higher magnification (inset) reveals these to consist of immunoreactive processes that occur clumped together (arrowheads).

Fig. 3.

The distribution of astrocytic cell bodies and processes show no inter-columnar differences within lamina 4C of the brain fixed with paraformaldehyde. Scale bar = 0.4 mm. A: A micrograph taken from a section near-adjacent to the one shown in Fig. 2 reacted for cytochrome oxidase histochemistry. This image was superimposed over a camera lucida drawing showing the positions of GFAP-immunoreactive processes. B: The camera lucida drawing is shown without the CO micrograph to facilitate visualization of the drawn GFAP-immunoreactive processes. Although the distribution of these processes is heterogeneous, the pattern does not align with the ocular dominance column stripes. C: A camera lucida drawing showing the position of GFAP-immunoreactive perikarya is shown superimposed over the

same CO micrograph shown in panel A. D: The camera lucida drawing alone shows a homogeneous distribution of GFAP-immunoreactive perikarya. Scale bar = 1 mm.

Fig. 4.

The distribution of β AR-like and GFAP immunoreactivity in V1 of a monocular monkey's brain fixed with acrolein. Scale bars = 1 mm. A: The distribution of β AR-li appears striped in lamina 4C (arrows). Alignment of this section with an adjacent one stained for CO using blood vessels 1, 2, and 3, among others, as fiduciary marks reveals that the darker bands match with the lighter CO bands in width and position. B: A portion of a camera lucida drawing mapping the GFAP-immunoreactive processes in a section adjacent to the CO section. Alignment of the GFAP and CO sections using the same set of fiduciary marks show no intercolumnar differences in the density of the processes, d and l: dark and light ocular dominance columns within lamina 4C as revealed by the CO stain. Circled asterisks mark the positions of blood vessels 1, 2, and 3.