L-Glutamate: A Neurotransmitter Candidate for Cone Photoreceptors in the Monkey Retina

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In order to examine whether L-aspartate and L-glutamate are photoreceptor transmitters in monkey retina, we have carried out two different studies: an autoradiographic localization of the high-affinity uptake sites for aspartate (Asp) and glutamate (Glu), and an immunocytochemical localization of the biosynthetic enzyme, L-aspartate aminotransferase (AAT). Our results show that (1) L-Glu is taken up by a sodium-dependent, high-affinity uptake system with a $K_m = 8 \pm 3.5 \times 10^{-6}$ M and a $V_{max} = 48 \pm 14 \times 10^{-12}$ mol/min/mg protein; (2) ^3H -L-Glu or ^3H -L- and D-Asp are taken up and accumulated by rod somata and inner segments in retinas incubated at 37°C or at ambient temperature; (3) cones accumulate ^3H -L-Glu at ambient temperature but not at 37°C. Neither ^3H -L-Asp nor D-Asp is accumulated by cones at either temperature; (4) rods and cones show the same labeling pattern irrespective of whether they are located in the central or peripheral retina; and (5) antiserum to AAT preferentially stains the cone somata, inner segments, and synaptic pedicles, while the outer segments are negative. Both foveal and peripheral cones are strongly reactive, but rods show little reactivity. These findings point to the existence of major differences between rods and cones in the uptake and metabolism of L-Asp and L-Glu and are consistent with L-Glu being a cone transmitter in the monkey retina.

The identity of the neurotransmitter used by vertebrate photoreceptors remains to be established. Recent studies indicate that among the known transmitter candidates, L-aspartate (L-Asp), L-glutamate (L-Glu), or both might function as transmitter for at least some photoreceptor cells. It has been known for some time that exogenously applied L-Asp or L-Glu causes depolarization of horizontal cells and off-center bipolars, while the on-center bipolars are hyperpolarized by these amino acids (Cervetto and MacNichol, 1972; Dowling and Ripps, 1972; Ishida and Fain, 1981; Kondo and Toyoda, 1980; Murakami et al., 1972, 1975; Negishi and Drujan, 1979; Shiells et al., 1981; Slaughter and Miller, 1981; Wu and Dowling, 1978). Although these observations suggest that L-Asp or L-Glu is the photoreceptor transmitter, the results have been questioned, since relatively high (mM) concentrations of the amino acids were required to elicit significant responses. Recently, however, L-Glu and its analogs have been shown to be effective at more phys-

iological (μM) concentrations when applied to dissociated horizontal and bipolar cells; at the same concentrations, L-Asp or D-Asp were found to be less efficient (Ishida, 1984; Ishida et al., 1983; Lasater and Dowling, 1982; Lasater et al., 1984).

A common property of aminergic and amino acid transmitter systems is the existence of a Na^+ -dependent, high-affinity system for transmitter re-uptake (Iversen, 1976). Unlike the acidic amino acid uptake systems in the brain, which do not distinguish between L-Asp and L-Glu (Balcar and Johnston, 1972; Fonnum, 1984; Logan and Snyder, 1971; McLennan, 1976; Wilkin et al., 1982), photoreceptors appear to possess a high-affinity uptake system for L-Asp and/or L-Glu, depending on the species examined. In human retina, while rods accumulate both amino acids, cones show much heavier labeling with L-Glu (Lam and Hollyfield, 1980). In guinea pig, rabbit, and pigeon retinas, cones but not rods accumulate D-Asp (Ehinger, 1981). In goldfish retina, red- and green-sensitive cones take up L-Asp and L-Glu, while rods accumulate L-Glu more readily than they do L-Asp (Marc and Lam, 1981).

L-Aspartate aminotransferase (AAT), an important enzyme in L-Glu metabolism, catalyzes the formation of L-Glu from α -ketoglutarate that is either produced in the neurons or transported from glial cells (Fonnum, 1984). The cytoplasmic form of this enzyme has been localized to presumed glutamate nerve terminals by immunocytochemical techniques, thus providing a useful histochemical marker for glutamergic neurons (Altschuler et al., 1981). Furthermore, in some, but not all, cases, antisera to AAT appear to stain cones selectively (Altschuler et al., 1982; Brandon and Lam, 1983).

We are interested in identifying the transmitter substances that are used by rod and cone photoreceptors in the monkey retina. In this communication, we report on our combined studies on the immunocytochemical localization of AAT and the autoradiographic localization of high-affinity uptake sites for L-Asp and L-Glu in the retina of the Old World Macaque monkey. Our findings suggest that L-Glu is a cone neurotransmitter in the monkey retina.

Materials and Methods

Normal adult monkeys (*Macaca nemestrina* or *M. fascicularis*, either sex) were deeply anesthetized, the eyes rapidly enucleated and immediately collected in chilled minimal essential medium (MEM) (Frederick et al., 1982). The globe was hemisected and the retina, along with the pigment epithelium, was dissected free. Three millimeter disks were punched out with a trephine, and were collected with a wide-bore pasteur pipette. Media were kept chilled throughout the course of these operations.

Uptake experiments

Two to three disks of retina were transferred to fresh MEM containing ^3H -L-Glu and unlabeled L-Glu at concentrations ranging from 1 to 100

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Figure 1. Kinetics of ^3H -L-Glu uptake. Three millimeter disks of monkey retina were incubated with varying concentrations of L-Glu for 10 min at 37°C . After rinsing, retinas were homogenized and the radioactivity accumulated was determined by scintillation counting. The figure represents an Eadie-Hofstee plot of the velocity of uptake (V) plotted against the ratio of the velocity (V) to the substrate concentration (S). L-Glu uptake could be described by a two-component system represented by the two straight lines. By extrapolation, the K_m were found to be $8 \pm 3.5 \times 10^{-6}$ and $3 \pm 1.1 \times 10^{-3}$ M and the $V_{\max}$ was estimated to be $48 \pm 14 \times 10^{-12}$ mol/min/mg protein and $1.5 \pm 0.8 \times 10^{-9}$ mol/min/mg protein for the "high"- and the "low"-affinity systems, respectively.

μM . After a 10 min incubation at 37°C , retinas were rapidly rinsed at 37°C for 2 min, and the disks homogenized in 0.4 N perchloric acid. After centrifugation of the homogenates, the supernatant was mixed with 10 ml Aquesol II and counted in a Beckmann scintillation counter (Sarthy and Lam, 1979). Protein was estimated by the method of Lowry et al. (1951) using BSA as the standard. For experiments on Na^+ dependence of uptake, Tris-hydrochloride (pH 7.4) was substituted for NaHCO_3 , and varying proportions of NaCl were replaced by sucrose.

To determine the amount of L-Glu accumulated during incubations, retinas exposed to ^3H -L-Glu were homogenized in 0.1 N HCl. The homogenate was centrifuged and the supernatant was concentrated to 50 μl . After mixing with nonradioactive L-Glu, the mixture was electrophoresed at 6000 V for 1.5 hr at pH 6.4. The paper was dried, and the location of Glu was detected by staining with ninhydrin. The spot was cut out, eluted in 0.1 N HCl for 1 hr, mixed with 10 ml Aquesol, and counted (Sarthy and Lam, 1979).

Autoradiography

Two to four disks of retina were transferred to small vials and preincubated in oxygenated MEM for 5 min at 37°C . ^3H -labeled L-Glu, L-Asp, or D-Asp (all purchased from New England Nuclear) were added to the incubation medium at a final concentration of 2–5 μM . Retinas were further incubated with gentle shaking for 10–30 min at 37°C . Using a wide-bore pasteur pipette, the disks were transferred to 10 ml fresh MEM and rinsed for 2 min at 37°C . Retinas were fixed in fresh, phosphate-buffered 2% glutaraldehyde for 4 hr at room temperature, dehydrated, and embedded in glycol methacrylate. Serial 2–3 μm sections were coated with Kodak NTB2 emulsion. After exposure for 1, 3, and 6 weeks, slides were developed in D19, fixed in Kodak Rapid Fix, and stained with azure II-methylene blue.

Immunocytochemical localization

Cytosolic AAT from porcine heart (Sigma) was further purified by column chromatography on Sephadex G-100 and CM-Sephadex, followed by preparative SDS-PAGE. The purity of the antigen was established from SDS-PAGE, in which only a single protein band with a $M_r = 45,000$ Da was obtained. Polyclonal antibodies were raised in rabbits by standard procedures. The specificity of anti-AAT was established

Figure 2. Na^+ dependence of L-Glu uptake. Disks of retina were incubated with 10 nM or 1 mM ^3H -Glu in media containing varying concentrations of Na^+ . After incubation at 37°C , retinas were rinsed and their radioactivity counted. The plot shows ^3H -L-Glu uptake (% control) as a function of $[\text{Na}^+]$, assuming the $[\text{Na}^+]$ in normal medium to be 100%.

from (1) immunodiffusion and immunoelectrophoresis tests, (2) an enzyme-linked immunosorbent test, and (3) a "Western" blot transfer experiment. This antiserum has also been used for immunohistochemical studies in rat liver, heart, kidney, cerebellum, and retina (Lin and Chen, 1983; Lin et al., 1983).

Eyes were obtained immediately after enucleation, hemisected, and immersed in a fixative of 4% paraformaldehyde containing either 0.34% lysine and 0.05% periodate (PLP) or 0.05% glutaraldehyde and 0.2% picric acid, both buffered with 0.1 M phosphate buffer, pH 7.3. Retinas were fixed for 90 min at 8°C and washed overnight in phosphate buffer containing 10% sucrose. Tissue fixed in glutaraldehyde was incubated in 1% sodium borohydride in phosphate buffer before processing for immunocytochemistry. Pieces of retina were sunk in 30% sucrose and frozen-sectioned at 25 μm . Free-floating sections were incubated for 36 hr at 8°C in anti-AAT diluted 1/150–1/300 in PBS containing 0.2% Triton and 1% goat serum. Some sections were washed, incubated for 45 min at 37°C in FITC-goat anti-rabbit IgG (diluted 1/30), mounted under glycerol, and examined using epifluorescence. Other sections were washed, incubated for 45 min at 37°C in goat anti-rabbit IgG, washed, and further incubated for 45 min at 37°C in rabbit antiperoxidase-peroxidase diluted 1/100. The peroxidase was localized by incubating sections for 8 min in 0.035% diaminobenzidine in 0.1 M Tris buffer, pH 7.2, containing 0.003% hydrogen peroxide. The sections were dehydrated, coverslipped, and examined with or without cresyl violet staining. Controls consisted of omission of primary antiserum or substitution of preimmune rabbit serum at the same concentration.

Results

Kinetics of L-Glu uptake

Before we began the autoradiographic localization of uptake sites, we examined kinetic properties of the L-Glu uptake system in the monkey retina. When disks of retina were incubated with micromolar concentrations of L-Glu, there was a rapid and linear accumulation of radioactivity for the first 10 min. The initial rate of ^3H -L-Glu uptake was found to increase linearly with increasing amounts of the amino acid in the incubation medium (Fig. 1). When uptake was expressed as an Eadie-Hofstee plot showing the velocity of uptake (V) against the ratio of velocity to substrate concentration (V/S), Glu uptake could be described by a two-component system whose kinetic parameters are, for the high-affinity system, an apparent $K_m = 8 \pm 3.5 \times 10^{-6}$ M and $V_{\max} = 48 \pm 14 \times 10^{-12}$ mol/min/mg protein; and, for the low-affinity system, $K_m = 3 \pm 1.1 \times 10^{-3}$ M and $V_{\max} = 1.5 \pm 0.8 \times 10^{-9}$ mol/min/mg protein. These values are similar to the K_m and $V_{\max}$ values for L-Glu in the rat retina (Neal and White, 1971, 1973; White and Neal, 1976).

Na⁺ dependence of uptake

The effect of Na⁺ removal on ³H-L-Glu uptake was investigated by measuring uptake in the presence of different concentrations of Na⁺ in the incubation medium. Furthermore, in order to examine the Na⁺ dependence of the two uptake systems, L-Glu uptake was measured at the concentrations of 10 nM and 1 mM. In Na⁺-free medium, ³H-L-Glu uptake was about 1–2% of the control value; increasing concentrations of Na⁺ in the medium resulted in a linear increase in the accumulation of ³H-L-Glu up to 50% [Na⁺] and did not vary much afterwards (Fig. 2). These results show that the high-affinity, L-Glu uptake system is Na⁺-dependent.

Autoradiographic localization of uptake

In retinas exposed to ³H-L- or D-Asp or ³H-L-Glu at 37°C, a large number of silver grains were located in the outer retina (Fig. 3, *A* and *B*). In peripheral retina, the majority of cell bodies in the outer nuclear layer (ONL) as well as their inner segments were heavily labeled. The outer segments, however, showed little uptake. At higher magnifications, the silver grains in the inner segment layer were found to be preferentially localized over the long and narrow inner segments of rods. The shorter, thicker cone inner segments were only lightly labeled. Furthermore, the cone somata, which line the scleral border of the ONL, also had few silver grains (Fig. 3, *E* and *F*). Since we could not obtain ³H-D-Glu, we were unable to examine its uptake pattern. The labeling pattern did not change for incubation times between 10 and 30 min. Biochemical analysis showed that at least 80% of ³H-labeled material accumulated during a 10 min incubation was L-Glu.

These results showed that while ³H-D- or L-Asp and ³H-L-Glu were readily taken up and accumulated by rods, the cones were essentially unlabeled. Our observations differed from the results of Brandon and Lam (1983), who had reported that in human and rat retinas, L-Glu labeled both rods and cones. These investigators, however, had carried out their experiments at “ambient” temperature, while our incubations had been performed at 37°C. In order to compare our results with those of Brandon and Lam, we repeated uptake experiments at room temperature (22 ± 2°C), and the results obtained are presented in Figure 3, *C*, *D* and *G*, *H*. With D-Asp, heavy labeling of both rod inner segments and somata was found, while cones showed relatively little labeling compared to rods. In the case of L-Glu, however, the distribution of grains over both rod and cone inner segments now was equally high, and significant amounts of label were also seen over the rod and cone somata (Fig. 3, *G* and *H*).

The effect of temperature on L-Glu uptake pattern is better demonstrated in tangential sections of retina. As shown in Figure 3, *I* and *J*, the large cone inner segments were found to be more heavily labeled in retinas incubated at room temperature than at 37°C. In our experiments with both ³H-Asp and Glu, labeled somata in the inner retina were located at the level of Muller nuclei. Also, the silver grains in the inner nuclear layer were not above background levels for either amino acid. As with peripheral retina, the cone-rich foveal region was markedly free of ³H-D-Asp label, but labeled photoreceptors appeared at its edge, where rods are first seen (Fig. 4, *A–E*).

Immunocytochemical localization of AAT

In the outer retina, the antiserum to AAT selectively labeled cells with the morphological characteristics of cone photoreceptors (Fig. 5, *A–E*). The cone somata, inner segments, axonal processes, and synaptic terminals were all reactive, but the outer segments remained unstained. In comparison to cones, the rods showed only light staining in the inner segment layer. Staining was heavy in the fovea, with every photoreceptor being labeled (Fig. 5*C*). In peripheral retina, individual stained cones were

separated by groups of unstained rods (Fig. 5*D*). The cone staining pattern remained consistent irrespective of whether localization was carried out using the indirect immunofluorescence technique (Fig. 5*E*) or the peroxidase–antiperoxidase method (Fig. 5, *A–D*), but there was a difference in AAT localization between fixatives. With the PLP fixative, the sections showed heavy staining of the axon and synaptic pedicle (Fig. 5*A*). The glutaraldehyde fixative resulted in heavy staining of the axon process, and the cone synaptic pedicle was often only lightly stained (Fig. 5, *A* and *B*). The cone inner segment and soma, however, were strongly immunoreactive for AAT with both fixatives. These results establish that AAT immunoreactivity is more highly concentrated in cones than in rods in the monkey retina.

In addition to cones, a number of neuronal cell bodies lying at the scleral edge of the inner plexiform layer were stained. From their location, these are likely to be amacrine cells. Furthermore, these cells had processes that terminated in two equally spaced bands in the inner plexiform layer (Fig. 5, *A* and *B*). The bands were particularly prominent in glutaraldehyde-fixed tissue. In addition to amacrine cells, cell bodies resembling bipolar somata were lightly stained (Fig. 5*B*). Last, some cell bodies in the ganglion cell layer lying next to the inner plexiform layer were immunoreactive for AAT.

Discussion

Several criteria have to be satisfied before a putative candidate can be accepted as a neurotransmitter at a particular synapse (as reviewed by Carpenter and Reese, 1981). In the present study, we have examined two aspects of neurotransmitter characterization, namely, localization of the biosynthetic enzyme and identification of the sites of high-affinity uptake.

Our immunocytochemical results show that, in the *Macaca* monkey retina, high levels of the biosynthetic enzyme AAT are found in the cone photoreceptors, while the rods contain relatively low levels. These findings are in agreement with the selective staining of cones by anti-AAT seen in guinea pig, rat, and human retinas (Altschuler et al., 1982; Brandon and Lam, 1983). Our data, however, are not consistent with the observations of Mosinger and Altschuler (1985), who recently reported that anti-AAT labeled rods but not cones in the rhesus monkey retina. Although the reason for this discrepancy is not known, differences in the AAT-antisera used may be critical. Our immunocytochemical data are also in agreement with the findings of Lowry et al. (1956), who reported that the highest levels of AAT activity were present in the inner segment region in microdissected monkey retinas. Further, they observed that the AAT activity was extremely low in the outer segment area, a result consistent with the absence of outer segment staining that we noted.

Our autoradiographic data show that L-Glu is accumulated by both rods and cones, while L- or D-Asp is accumulated only by rods—a result similar to that obtained with the human and rat retinas (Brandon and Lam, 1983). Further, the accumulation pattern for D- or L-Asp remained unchanged when the incubation was carried out at 20 or 37°C. In contrast, L-Glu accumulation varied with temperature. At 37°C, little accumulation was observed in cones, while at 20°C, uptake into cones was as strong as or stronger than that into rods. This difference in the labeling patterns between rods and cones may have various causes. The acidic amino acid uptake system present in cones may be selective for Glu and not for Asp at micromolar concentrations, indicating a real difference in pathways. A second possibility is that the rods and cones have the same uptake system and that the observed differences in labeling reflect intracellular metabolic differences. This explanation does not apply in the case of D-Asp, which is not readily metabolized in

Figure 3. Autoradiographic localization of ^3H -D-Asp (B, D, F, H) and ^3H -L-Glu (A, C, E, G, I, J) uptake in *Macaca* retina. Small disks of retina were incubated *in vitro* with micromolar concentrations of the amino acids at 37°C (A, B, E, F, J) or 20°C (C, D, G, H, I) for 10 min, washed briefly, and fixed in glutaraldehyde. Glycol methacrylate sections were processed for autoradiography using 2 week exposures as standard. The rods show marked uptake for Asp at both 37 and 20°C (B, D, F, H), with the inner segments and nuclei labeled heavily. Cones are lightly but distinctly labeled by Glu uptake at 20°C (C, G), but at 37°C (A, E) only occasional cone inner segments are labeled. Cones never label for Asp uptake under the conditions examined. This temperature difference in cone labeling is more clearly seen in tangential sections, comparing Glu uptake at 20°C (I) and 37°C (J). The black arrows mark cone inner segments and white arrows mark cone nuclei. The black arrowhead marks rod inner segments and the white arrowhead marks rod nuclei. ONL, Outer nuclear layer; INL, inner nuclear layer. A–D, $\times 291.4$; E–H, $\times 582.8$; I, J, $\times 728.5$.

the tissue. Hence, the lack of D-Asp accumulation in cones is likely to be due to a genuine absence of this uptake system from cones. It could, however, be argued that the Asp uptake system in cones may be inactivated when monkey retinas are maintained *in vitro*.

The accumulation of L-Glu at ambient temperature but not at 37°C may be due to a faster metabolism of the amino acid

at the higher temperature; and, unlike L-glutamate, the metabolites produced may not be retained during autoradiographic analysis. For example, at 37°C , the high levels of AAT present in cones may convert almost all ^3H -Glu taken up into α -ketoglutarate, a metabolite that cannot be fixed to tissue by glutaraldehyde. At 20°C , however, the enzyme, working under sub-optimal conditions, may spare some of the ^3H -L-Glu from

Figure 4. Autoradiograms of ^3H -D-Asp uptake in the foveal region of *Macaca* retina incubated *in vitro*. Panels *A* and *B* show light- and dark-field photographs of the entire section. The regions indicated in *A* are shown at higher power in *C–E*. Note that the fovea (*C*) contains almost no labeled structures except for two nuclei (arrows). The number of these dark-stained, small, and deeply placed nuclei that show ^3H -Asp uptake increases in more peripheral sections (*D*, *E*); these nuclei correspond to rod nuclei in position and staining characteristics. *isl*, Inner segment layer; *onl*, outer nuclear layer. *A*, *B*, $\times 27.9$; *C–E*, $\times 288.3$.

turnover. This would result in ^3H -Glu labeling of cones at 20°C but not at 37°C . If this explanation is correct, we would predict that inhibition of AAT activity should result in enhanced ^3H -L-Glu labeling of cones at 37°C . Experiments are under way to test this possibility.

Unlike these results in the outer retina, there was a marked difference in the pattern of ^3H -Asp or -Glu uptake and AAT staining in the inner retina. Immunocytochemical data showed the presence of AAT in a number of neuronal somata in the inner nuclear layer (INL), as well as in the ganglion cell layer. Furthermore, prominent bands of immunoreactivity were also found in the inner plexiform layer. In the autoradiographic studies with ^3H -Asp, only a light labeling of the inner retina was apparent, and the heavily labeled somata were probably those belonging to Muller cells, as judged from their location in the

INL of monkey retina. Similarly, with ^3H -L-Glu, although the background labeling of the tissue was higher, the heavily labeled cell bodies again appeared to be those of Muller cells. Thus, neither ^3H -Asp nor ^3H -Glu labeled the AAT-rich neurons in the inner retina. Incidentally, our localization of AAT in some bipolar somata is consistent with the suggestion of Slaughter and Miller (1983) that L-Glu also serves as a bipolar neurotransmitter in the retina.

When our autoradiographic and immunocytochemical data are considered together with those for human and rat retinas (Brandon and Lam, 1983; Lam and Hollyfield, 1980), it appears that in the monkey, human, and rat retinas, ^3H -L- or D-Asp is not accumulated by cones, while both Asp and L-Glu are readily taken up by rods. Furthermore, antisera to AAT stain the inner segment, cell body, and synaptic terminals of cone photorecep-

Figure 5. Immunocytochemical localization of antiserum to AAT visualized by peroxidase-antiperoxidase (A-D) and indirect immunofluorescence (E). A and B, Staining patterns found after fixation in paraformaldehyde without (A) and with (B) glutaraldehyde. Note that the inner segments (IS), cell bodies, and axons of the cones are stained equally well, but the synaptic terminal is stained only in A. The inner nuclear layer (INL) contains heavily labeled cell bodies in the general location of amacrine cells (large arrow, B) and more lightly labeled cell bodies resembling bipolar cells (small arrow, B). Other cells labeled are in the ganglion cell layer (GC). The inner nuclear layer is rich in AAT, with two bands marking sublaminae 2 and 4, especially after glutaraldehyde fixation. In the fovea (C), almost every photoreceptor is stained, while in more peripheral retina (D), AAT-labeled individual cones are separated by unstained rods. At higher power (E), after paraformaldehyde fixation, the entire cone is labeled except for the outer segment (OS) and nucleus (N). OPL, Outer plexiform layer; IPL, inner plexiform layer; GC, ganglion cell layer; SP, synaptic pedicle. A, B, $\times 400$; C, D, $\times 180$; E, $\times 900$.

tors in both human and monkey retinas (Brandon and Lam, 1983). These observations are, however, not in accord with results from other species. While Ehinger (1981) reported that ^3H -D-Asp was accumulated predominantly by the cones in guinea

pig retina, immunocytochemical studies (Altschuler et al., 1982) showed that anti-AAT staining was localized in the cone photoreceptors in this retina. Similarly, in pigeon and rabbit retinas, cones take up D-Asp (Ehinger, 1981). In the goldfish retina, red-

and green-sensitive cones take up L-Asp and L-Glu, while rods accumulate L-Glu more readily than L-Asp (Marc and Lam, 1981). The immunocytochemical localization of AAT in the pigeon, rabbit, and goldfish retinas has not been reported.

The definitive identification of the mammalian cone transmitter must await demonstration of its release specifically from cones, and its appropriate postsynaptic action on horizontal and bipolar cells. However, our findings of the presence of high levels of the Glu biosynthetic enzyme in cones but not rods and the existence of a selective, Na⁺-dependent, high-affinity uptake mechanism for L-Glu but not for L-Asp in cones alone are both consistent with L-Glu being a likely cone transmitter in the monkey retina.

References

- Altschuler, R. A., G. R. Neises, G. C. Harmison, R. J. Wenthold, and J. Fex (1981) Immunocytochemical localization of aspartate aminotransferase immunoreactivity in cochlear nucleus of the guinea pig. *Proc. Natl. Acad. Sci. USA* 78: 6553–6557.
- Altschuler, R. A., J. L. Mosinger, G. G. Harmison, M. H. Parakkal, and R. J. Wenthold (1982) Aspartate aminotransferase-like immunoreactivity as a marker for aspartate/glutamate in guinea pig photoreceptors. *Nature* 298: 657–659.
- Balcar, V. J., and G. A. R. Johnston (1972) The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. *J. Neurochem.* 19: 2657–2666.
- Brandon, C., and D. M. K. Lam (1983) L-Glutamic acid: A neurotransmitter candidate for cone photoreceptors in human and rat retinas. *Proc. Natl. Acad. Sci. USA* 80: 5117–5121.
- Carpenter, D. O., and T. S. Reese (1981) Chemistry and physiology of synaptic transmission. In *Basic Neurochemistry*, 3rd Ed., G. J. Siegel, R. W. Albers, B. W. Agranoff, and R. Katzmman, eds., pp. 161–181, Little, Brown, Boston.
- Cervetto, L., and E. F. MacNichol, Jr. (1972) Inactivation of horizontal cells in turtle retina by glutamate and aspartate. *Science* 183: 417–419.
- Dowling, J. E., and H. Ripps (1972) Adaptation of skate photoreceptors. *J. Gen. Physiol.* 60: 698–719.
- Ehinger, B. (1981) [³H]-D-Aspartate accumulation in the retina of pigeon, guinea pig, and rabbit. *Exp. Eye Res.* 33: 381–391.
- Ehinger, B. (1982) Neurotransmitter systems in the retina. *Retina* 2: 305–321.
- Fonnum, F. (1984) Glutamate: A neurotransmitter in mammalian brain. *J. Neurochem.* 42: 1–11.
- Frederick, J. M., M. E. Rayborn, A. M. Laties, D. M. K. Lam, and J. G. Hollyfield (1982) Dopaminergic neurons in the human retina. *J. Comp. Neurol.* 210: 65–79.
- Ishida, A. T. (1984) Responses of solitary retinal horizontal cells to L-glutamate and kainic acid are antagonized by D-aspartate. *Brain Res.* 298: 25–32.
- Ishida, A. T., and G. L. Fain (1981) D-Aspartate potentiates the effects of L-glutamate on horizontal cells in goldfish retina. *Proc. Natl. Acad. Sci. USA* 78: 5890–5894.
- Ishida, A. T., A. Kaneko, and M. Tachibana (1983) Solitary horizontal cells in culture. II. A new tool for examining effects of photoreceptor neurotransmitter candidates. *Vision Res.* 23: 1217–1220.
- Iversen, L. L. (1976) Inactivation of neurotransmitters. In *Synapses*, G. A. Cottrell and P. N. R. Usherwood, eds., pp. 137–153, Plenum, New York.
- Kondo, H., and J. I. Toyoda (1980) Dual effect of glutamate and aspartate on the on-center bipolar cells in the carp retina. *Brain Res.* 199: 240–243.
- Lam, D. M. K., and J. G. Hollyfield (1980) Localization of putative amino acid neurotransmitters in the human retina. *Exp. Eye Res.* 31: 729–732.
- Lasater, E. M., and J. E. Dowling (1982) Carp horizontal cells in culture respond selectively to L-glutamate and its agonists. *Proc. Natl. Acad. Sci. USA* 79: 936–940.
- Lasater, E. M., J. E. Dowling, and H. Ripps (1984) Pharmacological properties of isolated horizontal and bipolar cells from the skate retina. *J. Neurosci.* 4: 1966–1975.
- Lin, C.-T., and L. H. Chen (1983) Production and characterization of an antibody to cytosolic aspartate aminotransferase and immunolocalizations of the enzyme in rat organs. *Lab. Invest.* 48: 718–725.
- Lin, C.-T., H.-Z. Li, and J.-Y. Wu (1983) Immunocytochemical localization of L-glutamate decarboxylase, gamma-aminobutyric acid transaminase, cysteine sulfinic acid decarboxylase, aspartate aminotransferase and somatostatin in the rat retina. *Brain Res.* 270: 273–283.
- Logan, W. J., and S. H. Snyder (1971) Unique high affinity uptake systems for glycine, glutamic and aspartic acids in cerebral nervous tissues of the rat. *Nature* 234: 297–299.
- Lowry, O. H., N. R. Roberts, and C. Lewis (1956) The quantitative histochemistry of the retina. *J. Biol. Chem.* 220: 879–892.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
- Marc, R. E., and D. M. K. Lam (1981) Uptake of aspartic and glutamic acid by photoreceptors in goldfish retina. *Proc. Natl. Acad. Sci. USA* 78: 7185–7189.
- McLennan, H. (1976) The autoradiographic localization of L-[³H]-glutamate in rat brain tissue. *Brain Res.* 115: 139–144.
- Mosinger, J. L., and R. A. Altschuler (1985) Aspartate aminotransferase-like immunoreactivity in the guinea pig and monkey retinas. *J. Comp. Neurol.* 233: 255–268.
- Murakami, M., K. Ohtsu, and T. Otsuka (1972) Effects of chemicals on receptors and horizontal cells in the retina. *J. Physiol. (Lond.)* 227: 899–913.
- Murakami, M., T. Otsuka, and H. Shimazaki (1975) Effects of aspartate and glutamate on the bipolar cells in the carp retina. *Vision Res.* 15: 456–458.
- Neal, M. J., and R. D. White (1971) Uptake of L-[¹⁴C]-glutamate by rat retina. *Br. J. Pharmacol.* 43: 442–443.
- Neal, M. J., and R. D. White (1973) Multiplicity of transport systems for L-glutamate and L-aspartate in the retina. *Br. J. Pharmacol.* 49: 172–173.
- Negishi, K., and B. D. Drujan (1979) Effects of some amino acids on horizontal cells in the fish retina. *J. Neurosci. Res.* 4: 351–363.
- Sarthy, P. V., and D. M. K. Lam (1979) The uptake and release of ³H-dopamine in the goldfish retina. *J. Neurochem.* 32: 1269–1277.
- Shiells, R. A., G. Falk, and S. Nagh Shieh (1981) Action of glutamate and aspartate analogues on rod horizontal and bipolar cells. *Nature* 294: 592–594.
- Slaughter, M. D., and R. F. Miller (1981) 2-Amino-4-phosphonobutyric acid: A new pharmacological tool for retina research. *Science* 211: 182–185.
- Slaughter, M. D., and R. F. Miller (1983) Bipolar cells in the mudpuppy retina use an excitatory amino acid neurotransmitter. *Nature* 303: 537–538.
- White, R. D., and M. J. Neal (1976) The uptake of L-glutamate by the retina. *Brain Res.* 111: 79–93.
- Wilkin, G. P., J. Garthwaite, and R. Balazs (1982) Putative amino acid transmitters in the cerebellum. II. Electron microscopic localization of transport sites. *Brain Res.* 224: 69–80.
- Wu, S. M., and J. E. Dowling (1978) L-Aspartate: evidence for a role in cone photoreceptor synaptic transmission in the carp retina. *Proc. Natl. Acad. Sci. USA* 75: 5205–5209.