ent differences in fertilization ability in mixed pollination experiments. Paternity differences seen in this study are probably attributable to differential pollen transfer by insects rather than to gametic competition (S. Mazer, R. Burke, M. Stanton, unpublished data).
18. The patterns of fruit set and pollinator discrimination seen here resemble those observed in naturally occurring *R. raphanistrum* populations. At our primary study site in Hamden, Connecticut, insect visitors in both 1981 and 1983 made 64 to 65 percent of their visits to yellow flowers in color preference tests. In 1983, fruit set by yellows and whites (58.2 versus 55.1 percent) did not differ significantly and could not be increased by additional hand-pollination. In contrast, yellow-flowered plants did have significantly greater fruit set than white-flowered plants in 1981. Apparently, fruit set was limited by pollen in 1981, but not in 1983. These data suggest that pollinator discrimination can influence both maternal and paternal reproductive success, but only when pollen deposition is a limiting factor.
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Acetylcholine Receptor Synthesis in Retina and Transport to Optic Tectum in Goldfish

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Previous studies have suggested that the retinotectal system of the goldfish contains a nicotinic acetylcholine receptor (nAChR) that is sensitive to α-bungarotoxin. Extracellularly recorded field potentials elicited in response to visual stimulation can be blocked by α-bungarotoxin, and α-bungarotoxin can interfere with the maintenance of retinotectal synaptic connections. Whether the transmission between the retinal ganglion cells and the tectal cells is mediated by acetylcholine and whether nAChR's exist on the dendrites of tectal cells are questions that remain. The experiments described in this report were designed to determine the site of synthesis of the nAChR's associated with the goldfish retinotectal projection. Radioactive (^{35}S-labeled) methionine was injected into either the eye or the tectal ventricle, and the incorporation of radioactivity into the nAChR was measured by immunoprecipitation. The use of this technique provides evidence that an nAChR associated with the goldfish retinotectal projection is synthesized in the retina and transported to the optic tectum, which suggests a presynaptic site of acetylcholine action on retinal terminals.

A SUBSET OF MONOCLONAL ANTIbodies to nicotinic acetylcholine receptors (nAChR's) purified from the electroplaques of the electric ray *Torpedo californica* and the eel *Electrophorus electricus* (*1, 2*) interacts with antigens from the central nervous systems of the chick (*3*), frog (*4*), and goldfish (*5*). In goldfish, monoclonal antibodies seem to recognize an antigen that binds α-bungarotoxin, and antibody binding colocalizes with α-bungarotoxin binding in the layers of the optic projection (stratum opticum and superficial gray and white). We have used one of these monoclonal antibodies (mAb 47) (*2*) to study the site of synthesis of nAChR in the retinotectal projection of the goldfish *Carassius auratus*.

We found that cholinergic receptors are synthesized in the retina and transported to the optic tectum by rapid axonal transport. This result suggests that the ACh receptor is probably presynaptic and that it performs a modulatory function in retinotectal communication; the result may imply that the retinotectal transmitter is not, as has been suggested, ACh (*6–10*). In addition, this description of the synthesis and transport of an important modulatory molecule in the retinotectal system may advance the molecular characterization of retinotectal synaptogenesis. Finally, the metabolic labeling of neuronal nAChR's and detection by specific immunological precipitation described in this report will, in conjunction with molecular biological methods, allow the characterization of neuronal nAChR's and the definition of their relationship to α-bungarotoxin binding sites and neuromuscular AChR's.

Proteins were labeled by injection of [^{35}S]methionine into either the eye (8 μl, 73 μCi) or the tectal ventricle (2 μl, 18 μCi). After survival times appropriate for incorporation of [^{35}S]methionine into newly synthesized protein, the retina and individual optic tecta were removed and a detergent-solubilized membrane fraction was prepared in 50 m*M* 3-(*N*-morpholino)propanesulfonic acid (MOPS) and 1 m*M* EGTA, *p*H 7.4 (*11*). Aliquots of solubilized membranes were incubated for 1 hour at room temperature with appropriate volumes of mAb 47. The antibody-nAChR complex was precipitated with 35 μl of 10% cell suspension of *Staphylococcus aureus* membranes (incubation period of 1 hour with frequent mixing) by centrifugation for 5 minutes in a Microfuge. The pellet was washed three times and an aliquot of the resuspended pellet was assayed for radioactivity. The mAb 47 (and fixed *S. aureus* membranes) were used to specifically precipitate radioactivity incorporated into proteins with antigenic determinants in common with the nAChR from the *E. electricus* electroplaque. The precipitated radioactivity was identified as nAChR by two criteria (Fig. 1). (i) The precipitation could be inhibited by inclusion of affinity chromatography–purified nAChR from *Torpedo nobiliana*, and (ii) the dilution of antibody that precipitates half of the maximum amount of radioactivity is the same as that which precipitates half of the maximum amount of α-bungarotoxin binding activity from the goldfish retina and tectum and from *Electrophorus* electroplaque.

After radioactive methionine was injected into one eye, radioactivity was found in both tecta; however, the contralateral tectum contained at least four times as much radioactivity as the ipsilateral (Fig. 2). In addition, the specific radioactivity of total protein in the ipsilateral tectum was similar to that of skeletal muscle, which suggests that this represents the proportion of [^{35}S]methionine that leaked into the blood stream. (The projection of the retina to the optic tectum is totally contralateral in the goldfish.) Therefore, the difference in radioactivity between the contralateral and ipsilateral tecta represented the amount of radioactivity specifically transported to the tectum from the retina. Radioactivity was incorporated into nAChR in the optic tectum 6 hours after injection, and the radioactivity incorporated peaked 12 hours after injection (Fig. 2). This time course is compatible with fast axonal transport (*12*). Furthermore, the incorporation of radioactivity into the nAChR in the contralateral tectum

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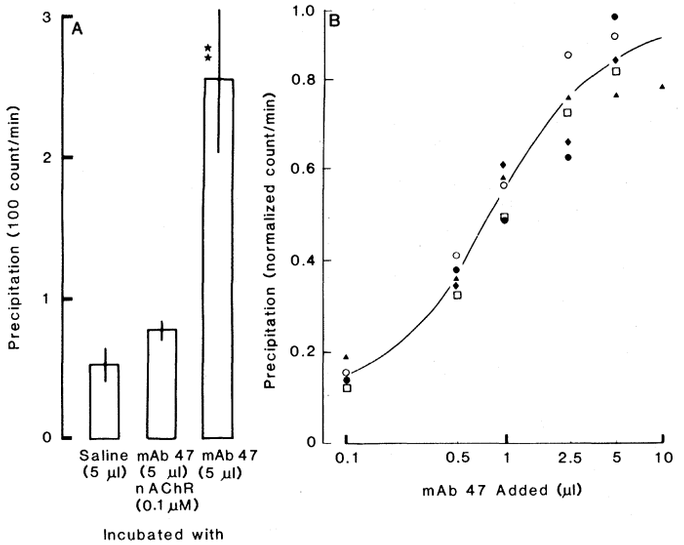

Fig. 1. (A) [^{35}S]Methionine precipitated from Triton X-100 solubilized retinal membranes by mAb 47. Fish were injected with [^{35}S]methionine 10 to 12 hours before an eye was removed. Portions of solubilized membranes were incubated for 1 hour at room temperature. Inclusion of *T. nobiliana* nAChR in the absence of mAb 47 did not affect the nonspecific radioactivity precipitated. The histograms shown are the means and SEM's of four to eight experiments (three to six fish per experiment). **Significantly different from other two conditions [*t* test with the probability corrected for multiple comparisons with a Bonferroni adjustment (*21*) ($P < 0.03$)]. (B) Dose-response relation of specific radioactivity precipitated versus volume of mAb 47 added under the following conditions: ▲, *Electrophorus*, α-[^{125}I]bungarotoxin; ●, goldfish retina, α-[^{125}I]bungarotoxin; ○, goldfish optic tectum, α-[^{125}I]bungarotoxin; □, goldfish retina, [^{35}S]methionine; and ◆, goldfish optic tectum, [^{35}S]methionine. The precipitated radioactivity was normalized to allow comparison between the different isotopes used.

could be totally blocked by crushing the optic nerve immediately before the injection or by injecting vincristine (an inhibitor of microtubule formation, 1 μ*M*) or cyclohexamide (an inhibitor of protein synthesis, 20 μg/ml) into the eye on the day of the injection. Injection of cyclohexamide (20 μg/ml) into the tectal ventricle completely inhibited incorporation of [^{35}S]methionine into nAChR in the optic tectum (methionine injected into the tectal ventricle) but had little effect on its incorporation into nAChR synthesized in the retina (retinal injection of methionine) and transported to the optic tectum. These experiments indicate that at least a portion of the nAChR associated with the retinotectal projection is synthesized in the retinal ganglion cells and transported to the optic tectum.

Between 30 and 40% of the α-[^{125}I]bungarotoxin binding in the optic tectum of the goldfish was lost in the week after the contralateral eye was removed, with a time course that corresponded to the loss of retinal terminals in the tectum (*6, 9*). Injection of [^{35}S]methionine into the tectum resulted in the incorporation of radioactivity into nAChR synthesized in the optic tectum. Binding of α-[^{125}I]bungarotoxin in the optic tectum and incorporation of [^{35}S]methionine into nAChR in the optic tectum (methionine injection into the optic tectum) 7 days after eye removal resulted in the loss of 34 ± 4% ($n = 4$) of the α-[^{125}I]bungarotoxin binding, but a slight increase of 11 ± 5% ($n = 4$) in the amount of [^{35}S]methionine incorporated into nAChR synthesized in the optic tectum. This result suggests that the portion of the nAChR lost after eye removal may not be synthesized in the optic tectum, and it provides further support for the hypothesis that a portion of nAChR is synthesized in the retina and transported to the optic tectum. Antibody precipitation was performed as described above, and α-[^{125}I]bungarotoxin binding (1 n*M* α-[^{125}I]bungarotoxin) was measured with an ammonium sulfate precipitation assay (*11*). Radioactivity in the contralateral tectum was normalized to that in the ipsilateral tectum (3005 count/min for α-[^{125}I]bungarotoxin and 1052 count/min for [^{35}S]methionine).

The data support previous conclusions (*13*) that the optic tectum of the goldfish contains an nAChR similar to those from the vertebrate neuromuscular junction and the electric organ of electric fish. The localization of the nAChR–α-bungarotoxin binding protein at the retinotectal projection has been suggested by the inhibition of visually induced field potentials (*7, 8*), loss of α-bungarotoxin binding activity in the layers of retinal projection in the optic tectum after eye removal (*6, 9*), loss of antigenic sites recognized by a polyclonal antibody preparation raised against *Electrophorus* nAChR after eye removal (*14*), and localization of these antigenic sites on optic nerve fibers (*14*). Whereas previous studies have provided evidence that a cholinergic receptor is associated with the goldfish retinotectal projection, the protein has not been exactly localized to the presynaptic retinal terminals or postsynaptic tectal dendrites. For example, the loss of antigenic sites after eye removal could be due either to a direct loss of presynaptic retinal terminals or to a concomitant loss of the postsynaptic density with the presynaptic terminal, as has been observed electron microscopically (*15*). The suggestion that nAChR's are present in the optic nerve was based on histochemical localizations to tissue that had been harshly fixed; in addition, the antibodies used could have contained contaminants (*14*). Our experiments provide evidence that an nAChR is synthesized in the retina and transported to the tectum to participate presumably as a

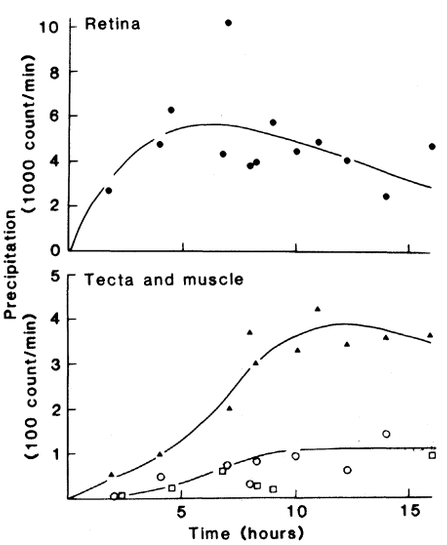

Fig. 2. Time course of [^{35}S]methionine incorporation into mAb 47 precipitable protein. Three fish, anesthetized in ice water, were intraocularly injected with 8 μl of [^{35}S]methionine for each point. The fish were killed after the appropriate intervals and the retina, ipsilateral and contralateral tecta, and skeletal muscle were collected. Portions of solubilized membranes (100 μl) were incubated for 1 hour at room temperature with either 2 μl of saline (control) or 2 μl of mAb 47 in duplicate. Antibody precipitation was performed as described. Specific [^{35}S]methionine incorporation for each tissue was calculated by subtraction of the no-mAb control. The points are ●, retina; ▲, contralateral tectum; ○, ipsilateral tectum; and □, skeletal muscle.

receptor on a presynaptic retinal terminal. These findings suggest that the retinal neurotransmitter may not be ACh, but rather that ACh modulates synaptic transmission in the visual system. This idea is supported by recent suggestions that the choline *O*-acetyltransferase present in the layers of retinal projection of the goldfish tectum is not lost after an eye is removed (*16, 17*). An attractive alternative for the cholinergic input to the optic tectum has been suggested by Gruberg and collaborators (*18, 19*). They have shown, in the turtle and the frog, that the nucleus isthmi projects to the optic tectum and that 90% of the choline *O*-acetyltransferase activity is lost after a bilateral lesion of the nuclei isthmi. One possibility is that fibers from the nucleus isthmi form nicotinic cholinergic axoaxonal synapses on retinal terminals and play a role in the control of retinotectal transmission. As suggested by Freeman (*10*) and Schmidt (*20*), the modulation of this receptor, now localized to the presynaptic terminal, may have important implications for the plasticity of synaptic connections in the goldfish visual system.

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Aminoguanidine Prevents Diabetes-Induced Arterial Wall Protein Cross-Linking

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Age-associated increases in collagen cross-linking and accumulation of advanced glycosylation products are both accelerated by diabetes, suggesting that glucose-derived cross-link formation may contribute to the development of chronic diabetic complications as well as certain physical changes of aging. Aminoguanidine, a nucleophilic hydrazine compound, prevented both the formation of fluorescent advanced nonenzymatic glycosylation products and the formation of glucose-derived collagen cross-links in vitro. Aminoguanidine administration to rats was equally effective in preventing diabetes-induced formation of fluorescent advanced nonenzymatic glycosylation products and cross-linking of arterial wall connective tissue protein in vivo. The identification of aminoguanidine as an inhibitor of advanced nonenzymatic glycosylation product formation now makes possible precise experimental definition of the pathogenetic significance of this process and suggests a potential clinical role for aminoguanidine in the future treatment of chronic diabetic complications.

CROSS-LINKING OF LONG-LIVED PROteins such as collagen increases as a function of age in both animals and man. In diabetes, the rate at which this age-associated increase in collagen cross-linking occurs is greatly accelerated (*1–4*). These observations have prompted a number of investigators to hypothesize that increased cross-linking of collagen and other extracellular matrix components may be responsible, in part, for the development of some of the physical changes that occur with normal aging and for some of the chronic complications of diabetes. The mechanism by which both diabetes and aging cause increased collagen cross-linking has not been elucidated, but recent work with model proteins suggests that advanced nonenzymatic glycosylation products could function as glucose-derived collagen cross-links (*5–10*).

The sequence of nonenzymatic glycosylation product formation that leads to cross-linking of proteins is shown in Fig. 1. Initially, glucose reacts with protein amino groups via nucleophilic addition to form a chemically reversible Schiff base adduct, which subsequently rearranges to the more stable but still chemically reversible Amadori product (*11–13*). Amadori products then slowly undergo a series of further reactions with amino groups on other proteins to form glucose-derived, intermolecular cross-links such as the recently characterized advanced glycosylation product, 2-(2-furoyl)-4(5)-(2-furanyl)-1*H*-imidazole (*9*, *13*). In contrast to early glycosylation products, which reach equilibrium levels after only 6 weeks, advanced glycosylation products continue to accumulate on long-lived proteins such as collagen over long periods of time (*13*). In collagen from diabetic individuals, this age-associated accumulation of advanced glycosylation products is accelerated by long-term exposure to elevated glucose concentrations (*14*).

Reasoning that glucose-derived protein cross-link formation would be prevented if reactive carbonyls on early glycosylation products could be pharmacologically blocked, we investigated the effects of a nucleophilic hydrazine compound, aminoguanidine (*15*, *16*), on this process (Fig. 1). In this report we describe experiments demonstrating that aminoguanidine inhibits advanced glycosylation product formation and glucose-derived collagen cross-linking in vitro. The results also show that aminoguanidine administered to rats inhibits diabetes-induced accumulation of advanced glycosylation products and abnormal cross-linking of arterial wall connective tissue protein.

The effect of aminoguanidine on advanced glycosylation product formation was evaluated by measuring specific fluorescence, as previously described for collagen (*14*). In each sample, incorporation of repurified ^{14}C-labeled glucose into acid-precipitable protein was also determined. Under these conditions, the values obtained for total glucose incorporation are essentially

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