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Retinoic acid has light-adaptive effects on horizontal cells in the retina

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ABSTRACT Ambient light conditions affect the morphology of synaptic elements within the cone pedicle and modulate the spatial properties of the horizontal cell receptive field. We describe here that the effects of retinoic acid on these properties are similar to those of light adaptation. Intraorbital injection of retinoic acid into eyes of dark-adapted carp that subsequently were kept in complete darkness results in the formation of numerous spinules at the terminal dendrites of horizontal cells, a typical feature of light-adapted retinae. The formation of these spinules during light adaptation is impaired in the presence of citral, a competitive inhibitor of the dehydrogenase responsible for the generation of retinoic acid *in vivo*. Intracellularly recorded responses of horizontal cells from dark-adapted eyecup preparations superfused with retinoic acid reveal typical light-adapted spatial properties. Retinoic acid thus appears to act as a light-signaling modulator. Its activity appears not to be at the transcriptional level because its action was not blocked by actinomycin.

Processing within the neuronal network of the retina is tuned by the ambient light conditions at several levels. In general this modulation helps to maintain high acuity, spatial resolution, and color vision over a broad range of intensities. Information about the ambient light condition is transmitted chemically within the retina, and there is evidence that the neurotransmitter dopamine is such a light signal. In almost all retinae, the levels of dopamine release increase with light adaptation. Dopamine mimics the effects of light in many instances, for example, it affects retinomotor movements, responsiveness of horizontal cells, the balance of rod–cone inputs to horizontal cells, electrical coupling between horizontal cells, and electrical coupling between AII amacrine cells (for a review see ref. 1). However, not all light-induced effects are attributable to dopamine, because some occur in dopamine-depleted retinae or are at least in part insensitive to dopamine antagonists (2–4). This has spurred the search for additional light-adaptive signals and nitric oxide (NO) has been suggested as a possible candidate. Although NO affects the responsiveness and coupling of horizontal cells, a direct link between NO and ambient light conditions has not yet been established (5, 6).

Recently it has been shown that in the mouse retina, retinoic acid (RA) is produced in pigment epithelium cells and that its formation is increased during steady background illumination (7). RA is a well known morphogenetic signaling molecule that can readily pass across membranes. Its light-correlated formation could make it an ideal light signal for the neuronal retina.

The formation of spinules is a well described phenomenon in the fish retina that is linked to light adaptation. Spinules are spine-like protrusions of the horizontal cell dendrites invaginating the cone pedicles and are part of the synaptic complex within the pedicles. Their formation is triggered by light adaptation, and their formation correlates with the appearance of color-opponent light responses in horizontal cells that are not present in dark-adapted retinas, where spinules are absent (8, 9). During light adaptation, the spatial organization of the receptive field of horizontal cells also undergoes typical changes, and the contribution of the center is markedly enhanced (2, 4).

Spinule formation and the spatial organization of the receptive field of horizontal cells in the carp retina therefore were used as parameters to analyze a potential involvement of retinoic acid during light adaptation.

We show here that all-*trans*-retinoic acid (at-RA) vigorously induces the formation of spinules at the terminal dendrites of horizontal cells in the carp retina and, in addition, mimics closely the effects of background light on the horizontal cell responses.

MATERIALS AND METHODS

All experiments were performed with carps of about a 25-cm body length. The animals were obtained through a local rearing station and kept in a large, aerated tank at about 22°C under a strict 12-h light/12-h dark cycle (lights on at 8 a.m. until 8 p.m.). After a minimum of 7 days in this tank, animals considered for experiments were transferred to a smaller tank and kept under the appropriate light conditions for each experiment.

Spinule Dynamics. The formation of spinules was quantified electronmicroscopically. The eyes were enucleated, hemisected, and fixed overnight at 4°C in 2.5% glutaraldehyde/1% paraformaldehyde/3% sucrose in 0.05 M phosphate buffer. Spinules were identified on the basis of their characteristic features, namely a finger-like extension of the terminal dendrite of horizontal cells within the cone pedicles with dense material lining its tip and part of the shaft. The number of spinules within a given section of a cone pedicle was counted and related to the number of synaptic ribbons within this pedicle (8). This spinules per ribbon (spr) value was determined for a minimum of 30 pedicles in the ventral and dorsal part of each retina, in three to six animals. Mean values and standard deviation are given, significance was calculated by using the Wilcoxon, Mann–Whitney test. Drugs were injected in a volume of 10 μl into the vitreous of the right eye by using a Hamilton syringe. The left eye received an injection of only the vehicle and served as a control. at-RA and citral (3,7-dimethyl-2,6-octadienal) were dissolved in dimethyl sulfoxide (DMSO). The given concentrations are estimated intraorbital concentrations based on the concentration within the injected volume and the size of the eyeball. All chemicals were obtained through Sigma.

Immunoblotting. The retinae were homogenized in ice-cold buffer (20 mM Tris·Cl, pH 7.4/10 mM $MgCl_2$/0.6 mM $CaCl_2$/0.5 mM EGTA/0.005% Triton X-100/1 mM phenylmethylsulfonyl fluoride/2 μg/ml leupeptin/5 μg/ml aprotinin) by sonication. Protein concentrations were determined according to the method of Bradford. Samples (60 μg of protein) of the retinal homogenates were prepared for SDS/PAGE and separated on 10%

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Abbreviations: RA, retinoic acid; at-RA, all-*trans*-RA; spr, spinules per ribbon.

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linear gels, and proteins were blotted onto nitrocellulose. The complete transfer of proteins was always controlled by reversible staining of the blots with Ponceau S and staining of the gels with Coomassie brilliant blue R250. The blots were washed in Tris-buffered saline, nonspecific binding was blocked (5% nonfat powdered milk in TBS, 37°C, 1 hr), and the blots were further incubated either with anti-rod transducin ($G\alpha t_1$, Santa Cruz Biotechnology, SC-389, diluted 1:200 in TBS) or with anti-c-jun (c-jun/AP-1, Oncogene, PC06, diluted 1:100 in TBS) at 4°C overnight. After three washes (10 min each), the blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgGs (Bio-Rad, diluted 1:3,000 in TBS/2% powdered milk, room temperature, 2 hr) and washed again, and immunoreactive proteins were detected by using the chemiluminescence method (ECL, Amersham) and Kodak X-O-Mat autoradiographic films. The amounts of the immunoreactive proteins were measured by densitometric scanning and by determining the curve area minus background.

Electrophysiology. Intracellular recordings with sharp electrodes were made from an inverted eyecup preparation. The eye was enucleated and hemisected in dim, red light. The eyecup was inverted gently and placed on a wooden dome of appropriate size. The eyecup was fixed along its exterior rim on the dome with layers of tissue paper that also served for subsequent drainage. The outlet of the superfusion system was placed with a micromanipulator close to the apex of the inverted retina, and the flow of the superfusate (1 ml/min) produced a film of about 300–500 μm over the retina. The Ringer superfusate consisted of 102 mM NaCl/2.6 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$/5 mM D-glucose/28 mM $NaHCO_3$ and was continuously aerated with carbogen (95% O_2/5% CO_2) to maintain a pH of 7.4. The recording technique and data storage have been described previously (6). The light responses of horizontal cells were identified based on their stimulus-dependent characteristics, and in some instances the origin was confirmed by Lucifer Yellow injections.

RESULTS

Light adaptation of the carp retina is accompanied by extensive structural changes within the cone pedicle. The terminal dendrites of the cone horizontal cells invaginate deeper into the cone pedicle and disperse more radially within the pedicle. At the same time they form numerous spinules that protrude into the cone pedicle. Spinules are not formed along the membrane of the dendrite facing the synaptic ribbon. Spinules are typically about 0.5 μm in length, 0.1 μm wide, and their tip and part of their shaft are lined with prominent membrane densities (9). In control experiments, animals were dark-adapted at 9 a.m. for 180 min and then subsequently light-adapted for 45 min. Cross-sections of cone pedicles revealed the typical arrangement of horizontal cell dendrites of a light-adapted retina (Fig. 1*A*). In addition, numerous spinules were formed and the spr value was 2.51 ± 0.51 (n = 6 animals; Fig. 1*D*). In another series of experiments, after 180 min of dark adaptation the right eyes of the animals received an injection of at-RA resulting in an estimated intravitreal concentration of 0.5 μM. The left eyes received an injection of the vehicle DMSO (0.25%). The injections were made under dim red illumination (about 4 $\mu W/cm^2$) and were completed within 1–2 min after which the animals were allowed to swim for 45 min in

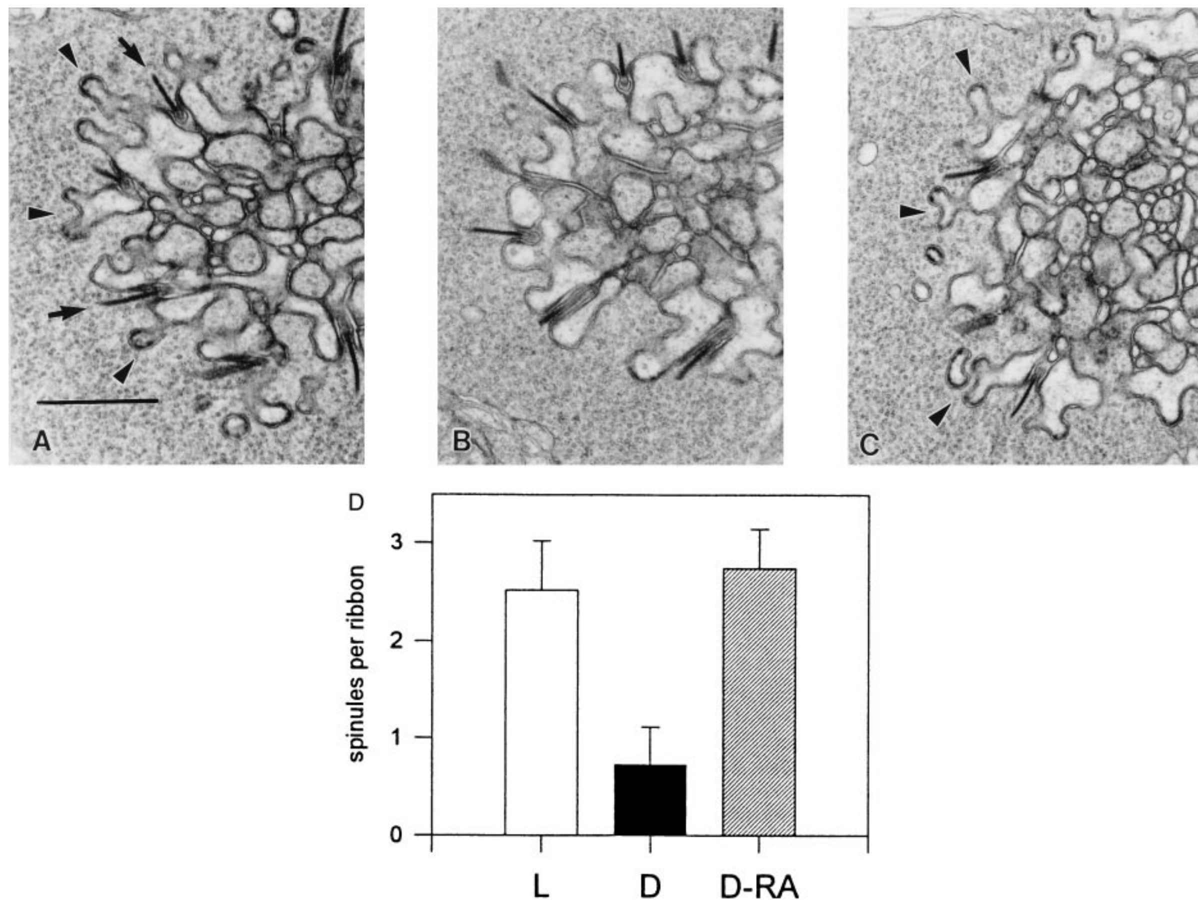

FIG. 1. Effect of at-RA on spinule formation. (*A–C*) Electron micrographs of tangential sections at the level of the outer plexiform layer of the carp retina. Part of a cone pedicle with ribbon synapses (arrows) is shown in each micrograph. (Bar = 1 μm.) (*A*) Numerous spinules (arrowheads) are visible in a light-adapted control. (*B* and *C*) Micrographs from the left and right eyes of the same dark-adapted animal. Spinules are not present in the untreated retina (*B*) but are present in the retina treated with at-RA (*C*). (*D*) Quantification of the spinule formation reveals that injection of at-RA into the eyes of dark-adapted animals (D-RA; n = 5 animals) results in a number of spinules significantly different from the number of spinules in the untreated eyes (D) and similar to that obtained by light adaptation (L).

complete darkness before they were decapitated in dim red light. The eyes were enucleated, hemisected, and transferred into the fixative in dim red light and subsequently stored in complete darkness. The exposure of the retina to dim red light during this procedure was kept minimal and never exceeded 2 min. A comparison of tangential sections through the cone pedicle of the control and injected eyes revealed striking differences. Whereas the arrangement of horizontal cell dendrites in the control retinae was typical for a dark-adapted retina (Fig. 1*B*), the retinae of the injected eye displayed a morphological arrangement within the cone pedicle that is typical for a light-adapted retina (Fig. 1*C*). Spinules were almost absent in the dark-adapted controls, and the spr value was 0.72 ± 0.39. In the dark-adapted retinae, incubated with at-RA, spinules were very prominent and the spr value was 2.74 ± 0.41 (n = 5; Fig. 1*D*). Both the overall pedicle morphology and the number of spinules of the at-RA-injected retinae were indistinguishable from a light-adapted retina, indicating that at-RA was able to mimic these light-induced characteristics of the synaptic complex within a cone pedicle. Similar results were obtained with concentrations of at-RA ranging from 10 nM to 1 μM, with an optimal response at 0.5 μM. Freshly prepared at-RA was effective in all 15 experiments, but at-RA that was partially bleached by extended exposure to room light was ineffective.

The effect of at-RA was restricted to the synaptic complex within the cone pedicles. The synaptic complex within the rod spherule was not affected. The terminal dendrites of rod horizontal cells do not form spinules after light adaptation, and, in line with this, at-RA also did not induce the formation of any spinule-like protrusions nor did it affect the arrangement of dendrites within the spherule (Fig. 2).

The drug citral, a competitive inhibitor of aldehyde dehydrogenase, has been used to inhibit the synthesis of retinoic acid in epithelial cells, in gastrulating *Xenopus* embryos, in the developing zebra fish retina, and in the embryonic mouse retina (7, 11–14). Citral at a concentration of 200 μM was injected into the right eyes of animals that had been dark-adapted for 180 min. The injection was done under dim red light as described above for at-RA. After the injection the animals were allowed to swim for 25 min in complete darkness. Then the tank was illuminated with a 50-W halogen lamp for 60 min. The fish subsequently were decapitated and enucleated, and the eye was hemisected and transferred to the fixative. Examination of the control retina revealed the presence of numerous spinules (Fig. 3*A*) and the spr value was 3.12 ± 0.68 (n = 5; Fig. 3*C*). The retinae injected with citral showed an overall morphology distinctive of that of a light-adapted retina (Fig. 3*B*). There was a tendency of centralization of the large, horizontal cell profiles within the cone pedicle, which is typical for a dark-adapted retina. Furthermore, the number of spinules was significantly reduced. The spr value was 1.91 ± 0.53 and significantly different from the control value ($P < 0.01$; Fig. 3*D*). In many instances, however, spinule-like extensions from the horizontal cell profiles were visible that were lacking the spinule-typical membrane densities. This might suggest that citral is particularly affecting a process responsible for the formation of these membrane densities. It has been shown already that the formation of spinules comprises two or more steps that are partially independent from each other (15). Citral also enhanced the frequency of round-shaped, electron-lucent profiles within the cytoplasma of the pedicle. Their frequency varied from preparation to preparation, and they might be vacuoles blebbed off from the horizontal cell dendrites during some adverse reaction. The citral-induced inhibition of spinule formation could be rescued completely by the subsequent injection of 0.5 μM at-RA (Fig. 3 *C* and *D*). The number of spinules returned to normal values, the overall preservation of the tissue, however, was impaired, and the number of vacuoles had increased. There was no significant difference between the inhibitory effect of citral on spinule formation between the dorsal and ventral retina.

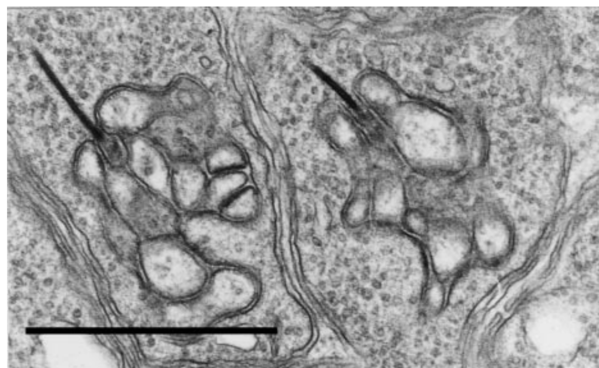

FIG. 2. Electron micrograph of rod spherules of a dark-adapted retina that was treated with at-RA. Horizontal cell dendrites of rod horizontal cells do not show any spinule-like protrusions. (Bar = 1 μm.)

The effect of at-RA on spinule formation showed a time course similar to that of light adaptation. After 15 min of incubation the spr value was 1.15 ± 0.51 and significantly ($P < 0.01$) different from the control value (0.89 ± 0.24; n = 3). The spr values increased over the next 25 min and reached a plateau at 40 min. Assuming that the diffusion of at-RA from the site of injection to the site of action at the outer plexiform level of the retina takes some time, a rather rapid effect within a few minutes of at-RA seems likely.

It is well established that at-RA acts at the transcriptional level (for a review see ref. 16). The rapid onset of the effect of at-RA on spinule formation does not match with the time course of its reported effects during embryogenesis and development, although a direct comparison is difficult because of the different experimental approaches. Therefore, we used actinomycin to block transcriptional activity to obtain further insight into the signaling pathway. After 180 min of dark adaptation, actinomycin (200 μM) was injected into the vitreous of right eyes (n = 2) under dim red light. After 15, 60, or 120 min in the dark, both eyes were injected with 0.5 μM at-RA under dim red light. The animals subsequently were kept 45 min in the dark and then decapitated and enucleated under dim red light. Both eyes were hemisected and fixed in the dark. The cone pedicles of retinae of both eyes showed light-adapted characteristics, and the spr values were typical for light-adapted retinae and identical for both eyes (Fig. 4*A*). This result was obtained regardless of whether the injection of actinomycin was done 15, 60, or 120 min before the injection of at-RA.

To exclude the possibility that the lack of an effect of actinomycin resulted only from its inability to diffuse into the retina, we performed a positive control experiment. We examined the effect of actinomycin on the transcription products of two genes that are known to be continuously expressed and also show a fast transcriptional regulation. One is the c-jun protein and the other, rod-transducin. Injection of 200 μM actinomycin was performed as described above, and after 120 min the animals were killed and the retinae were homogenized. The injection of actinomycin resulted in changes in the amounts of both proteins compared with the control eyes that received only an injection of the vehicle (Fig. 4*B*). In the case of the rod-transducin immunoreactive protein, actinomycin caused a mean reduction of 36% ± 24% in three independent experiments. The amount of c-jun, in contrast, was increased significantly in the same preparations by 229% ± 156%. These data indicate that actinomycin was able to affect the transcription of retinal genes in the same *in vivo* incubation as used for the spinule experiments.

Light adaptation affects not only the terminal dendrites of horizontal cells but also the spatial characteristics of the light responses of horizontal cells. After light adaptation, the responses to small spot stimuli in the receptive field center are enhanced and

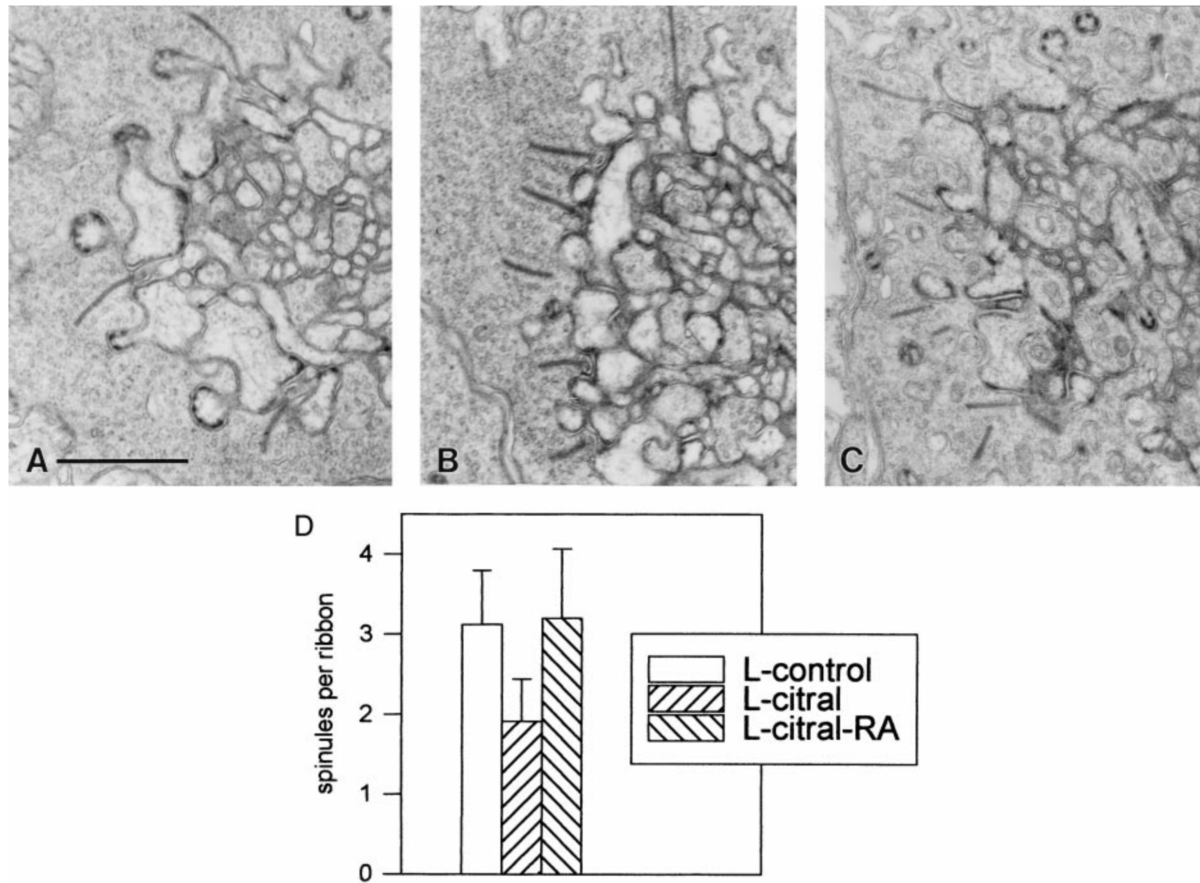

FIG. 3. Effect of citral on light-induced spinule formation. Electron micrographs of the control retina (*A*) of the retina that was treated with citral (200 μM) before light adaptation (*B*) and of a retina that, after being treated with citral, received an injection of 0.5 μM at-RA (*C*). (Bar = 1 μm.) Quantitative analysis (*D*) revealed a significant ($P < 0.01$; $n = 5$ animals) inhibition of spinule formation (L) by citral (L-CIT) and rescue of spinule formation by subsequent injection of at-RA ($n = 2$ animals).

the responses to annuli often are reduced (3, 4, 17, 18). Therefore, we used intracellular recordings to monitor possible effects of at-RA on the spatial characteristics of horizontal cell light responses.

Everted eye cup preparations were obtained from dark-adapted animals and superfused continuously with Ringer. In an initial set of experiments, the preparation was kept in darkness for an additional 30 min. The electrode penetration then was performed in complete darkness. Penetration of a horizontal cell was assumed after a voltage drop of about 30–40 mV. Nonsaturating white-light stimuli (spot, 0.9-mm diameter; annulus, 1.1-mm inner diameter and 3.2-mm outer diameter) of 500-ms duration were applied. The light responses of an H1 horizontal cell to these stimuli at an intensity of log −3 are shown in Fig. 5*A Top*. The ratio of the response amplitude to annular stimulation and the response amplitude to spot stimulation (A/S ratio) in this case was 2.59. The A/S ratio obtained from seven cells was plotted against the intensity (Fig. 5*B*, solid circles). In another set of experiments, the preparation was illuminated for 10 min with a diffuse white background light of log −3 intensity. After about 20 min the electrode penetration was performed as above and light responses obtained from an H1 cell are shown in the middle traces of Fig. 5*A*. The A/S ratio was 0.29, and the A/S ratio of seven cells was plotted against the intensity (Fig. 5*B*, open circles). In a third set of experiments, the preparation was superfused for 10 min with a Ringer containing 100 μM at-RA in complete darkness. The relatively high concentration of at-RA was necessary because, due to its lipophilic character, at-RA remained mainly at the upper, outer surface of the liquid film formed by the superfusate and was continuously washed out. The actual concentration that reached the retinal surface and finally the site of action at the outer plexiform level might be considerably smaller. After about an additional 20 min in darkness, the electrode penetration and recordings were made as in the other experiments. Light responses of an H1 cell are shown in the bottom traces of Fig. 5*A*. The A/S ratio was 0.13, and the A/S ratio of seven cells was plotted against intensity (Fig. 5*B*, triangles).

Ten minutes of light adaptation and 10 min of superfusion with at-RA in darkness had almost identical effects on the A/S ratio. Both treatments decreased the A/S ratio because of a considerable increase in the spot response and a simultaneous decrease in the annulus response. The observed dependence of the A/S ratio on the light intensity, in particular, its increase with increasing intensity was maintained after at-RA-superfusion. The match of the curves obtained after light adaptation and after at-RA superfusion strongly suggests a link between the two.

DISCUSSION

The formation of spinules at the terminal dendrites of horizontal cells in the teleost retina is an example of short-term sensory experience imposing structural synaptic changes. The underlying signal cascade of this structural synaptic plasticity is only partially known. It has been shown that activation of protein kinase C by phorbol esters induces the formation of spinules and that, among several other proteins, the growth-associated protein GAP 43 is a likely candidate for phosphorylation (19, 20). The involvement of cAMP in the process is still controversial. In the *in vivo* situation cAMP is without effect (21), whereas in the *in vitro* situation it can induce the formation of spinules (22). Similarly, the neurochemical signal that triggers spinule formation has not yet been fully determined. Dopamine, which is released from interplexiform cells (23, 24), is a likely candidate, but its effect on

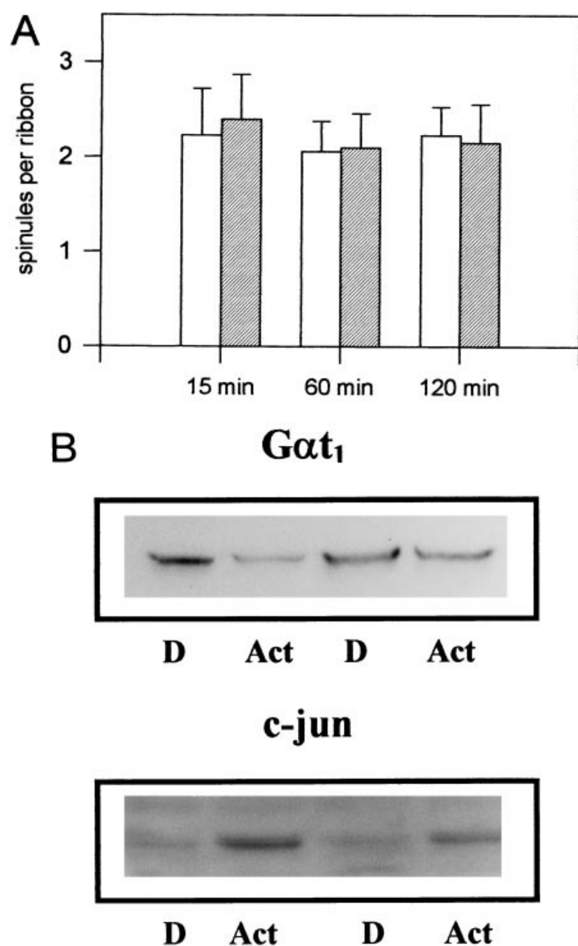

FIG. 4. *In vivo* effect of actinomycin on at-RA induced spinule formation. (*A*) Preincubation (15, 60, and 120 min) with actinomycin (200 μM) did not block the effects of at-RA (0.5 μM). Open bars represent the data from dark-adapted control retinae that received an injection of at-RA, and hatched bars represent data from the retinae that in addition were preincubated with actinomycin ($n = 2$ animals for each time point). (*B*) *In vivo* effect of actinomycin on the expression of rod transducin Gαt$_1$ and c-jun in the carp retina. Immunoblots of SDS/10% PAGE gels on which aliquots (60 μg of protein each) of homogenates of dark-adapted controls (D) and actinomycin-treated retinas (2 hr *in vivo* preincubation; Act) were separated. The expression patterns of a 39-kDa anti-Gαt$_1$- and a 39-kDa anti-c-jun-immunoreactive protein are shown.

spinule formation never matches that of light. There are controversial reports about the role of NO that is localized in luminosity-type cone horizontal cells (5, 6, 25, 26).

This study reports that at-RA is a very powerful signal for spinule formation. Its time course is similar to that of light, and the spr value is of the same magnitude as for light or even sometimes slightly higher. It was effective in all experiments over the tested concentration range, and it not only induced the formation of spinules but also induced other characteristics of the cone–pedicle complex that are typical for light-adapted retinas. Moreover, it was possible to demonstrate the involvement of at-RA in spinule formation during light adaptation. Citral, a competitive inhibitor of the enzyme aldehyde dehydrogenase responsible for the *in vivo* production of at-RA, impaired the formation of spinules during light adaptation. In particular, the deposition of densities was affected, underlining the notion that spinule formation includes several, partially independent processes (8).

There are at least three retinoic acid-generating enzymes in the eye, and their distribution undergoes developmental changes (for a review see ref. 27). In the zebra fish retina, the dehydrogenase located in the ventral retina is more sensitive to competitive inhibition by citral than that in the dorsal retina, and citral can prevent the development of a ventral retina (13, 14, 28). We did not find a significant difference between the inhibitory effect of citral on spinule formation in dorsal and ventral retina. It may be that the concentration of citral was high enough to affect both enzymes or that only one type of dehydrogenase is present in the adult carp retina. In addition to retinal dehydrogenases, there is also a dehydrogenase located in the pigment epithelium that does not show a dorsoventral asymmetry. In the postnatal mouse the relative RA amount released from the pigment epithelium is about 90% compared with 10% from the neural retina. This indicates that the pigment epithelium might be the likely source of at-RA generation during light adaptation. This idea is supported by the finding that light increases RA accumulation in the neural retina if the retina and the pigment epithelium are left together (7). A likely explanation for this observation is that the dehydrogenase in the pigment epithelium converts some of the chromophore all-*trans*-retinaldehyde released from bleached rhodopsin and transported into the pigment epithelium (7). The oxidation of at-RA by the dehydrogenase is an irreversible reaction, and the generation of at-RA thus is an unavoidable byproduct of the bleaching process. This byproduct necessarily correlates with the amount of light exposure, particularly with light intensity and duration. at-RA produced in the pigment epithelium therefore would be a reliable signal for the ambient light condition.

In addition to the pigment epithelium, there are other sources within the retina that produce at-RA. Its synthesis has been measured directly from Müller cells of the rabbit retina (29), and its synthesizing enzyme, aldehyde dehydrogenase, has been localized in amacrine cells of the bovine retina (30, 31). Furthermore, Müller cells and amacrine cells in several species show immunoreactivity for the at-RA-binding protein, indicating an involvement of these cells in at-RA metabolism (32, 33).

The localization of at-RA in cells of the inner retina, together with the existence of other modulators, might partially explain why light-adaptive changes such as spinule formation can be observed in isolated retinas, deprived of the pigment epithelium (34). Our own experience and that of others (35), however, has shown that formation of spinules is much more difficult and sometimes impossible to obtain in such preparations, whereas the formation is reproducible and reliable in preparations in which the pigment epithelium is left together with the retina.

A light-signaling role for at-RA also is supported by the effect of at-RA on the spatial properties of horizontal cells. The ratio of the light responses to annular and spot stimulation is used frequently to describe the spatial organization of the receptive field of horizontal cells. It has been demonstrated that this ratio is affected by background light (2, 4, 17, 18, 36–38). We have shown here that the effects of background light are matched perfectly by the effects of at-RA. Until recently, dopamine has been considered to be the light-signaling transmitter for most of the observed light-adaptive effects, but there are reports that conflict with this view (2, 4) and suggest that dopamine is not the only signal for steady-light conditions. Indeed, release studies have shown that steady light does not enhance the release of dopamine in the fish retina (39). The generation of at-RA, on the other hand, increases with steady light (7) and therefore could signal this light condition.

At present we do not have an explanation for the mechanisms downstream of the generation of at-RA that could explain its effects on horizontal cells. The well established effects of at-RA at the transcriptional level, including the activation of nuclear receptors and RA response elements, do not seem to play a major role. First, the time course of the at-RA action is rather short, and second, blocking transcription with actinomycin did not inhibit the effects of at-RA. The positive control experiments excluded the possibility that actinomycin did not reach the horizontal cell level after intraocular injection. It affected the transcription of the

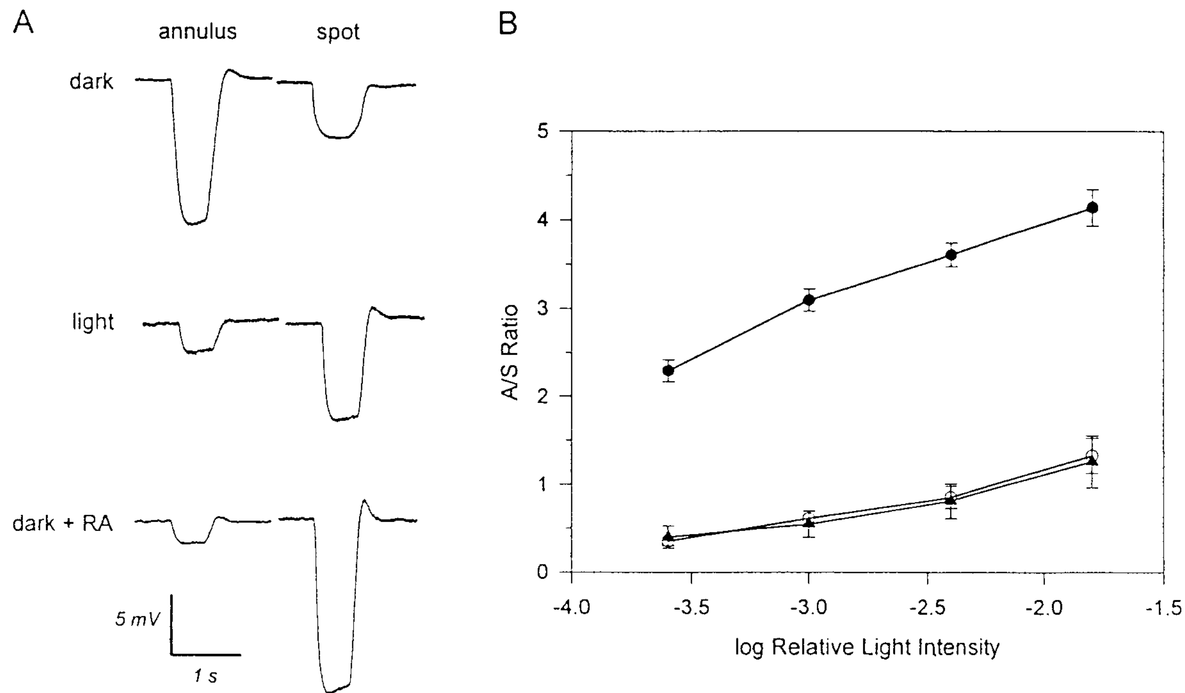

FIG. 5. Effects of at-RA on the spatial properties of the light responses of H1 horizontal cells. (*A*) Light responses to annular and centered spot stimulation at an intensity of log −3 under the indicated conditions. (*B*) Plot of the annulus/spot ratio against light intensity. Solid circles represent the data obtained from a dark-adapted retina, and open circles represent data from a light-adapted retina. Solid triangles represent the data obtained from a dark-adapted retina superfused with at-RA.

rod-transducin gene in similar experiments, and for doing so it had to pass beyond horizontal cells. An *in vivo* ocular injection of actinomycin also was used to study the cytidine metabolism in the rat retina, where it blocked transcription to a large extent (40), indicating again that the *in vivo* injection of actinomycin is a reliable method for studying transcriptional events.

One explanation for the effects of at-RA might be that it enhances the release of endogenous dopamine, and we are currently investigating this possibility. However, because at-RA is a much stronger signal with a faster time course than dopamine, a direct effect of at-RA upon protein kinases appears more likely (41, 42). We have shown previously that protein biosynthesis is not involved in spinule formation during the first 45 min and that protein phosphorylation is the major mechanism (19).

Retinoic acid has numerous pharmaceutical applications, and in several of them the signal-transduction pathway is obscure. The robust effect of at-RA on spinule formation might offer a chance to unravel the mechanisms of possible nontranscriptional effects of RA.

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