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Factors Affecting Release of ^{3}H-Dopamine from Perfused Carp Retina

Patricia O'Connor, Sara J. Dorison, Keith J. Watling,[1] and John E. Dowling

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

The effects of putative retinal neurotransmitters and several neuropeptides on ^{3}H-dopamine release from isolated perfused carp (*Cyprinus carpio*) retinas were studied. Of the transmitter candidates tested, only serotonin (5-HT) and the 5-HT agonist tryptamine released ^{3}H-dopamine. However, the release evoked by these agents was calcium (Ca^{2+}) independent and not blockable by the 5-HT antagonist methysergide. We also investigated the antagonism of inhibitory inputs as a potential regulatory mechanism for dopamine release and found that the GABA antagonists, bicuculline and picrotoxin, stimulated a dose-dependent release of ^{3}H-dopamine. The effects of the GABA antagonists were dependent on extracellular Ca^{2+} and could be inhibited by perfusion of the retina with GABA. Bicuculline and picrotoxin also stimulated an increase in cAMP accumulation, which was inhibited by the dopamine antagonist haloperidol. Our results support the hypothesis that the dopaminergic interplexiform cells of the teleost retina are under GABAergic inhibitory control.

Dopamine is the principal catecholamine in the vertebrate retina (Ehinger, 1976). In teleost fish, retinal dopamine appears to be confined to the interplexiform cells, neurons that receive their input from amacrine cells in the inner plexiform layer and send the majority of their output to horizontal and bipolar cells in the outer plexiform layer (Dowling and Ehinger, 1978). Dopamine has been shown to stimulate adenylate cyclase activity in the fish retina (Dowling and Watling, 1981; Watling and Dowling, 1981) and in isolated retinal horizontal cells (Van Buskirk and Dowling, 1981). In addition, dopamine has been reported to decrease light responsiveness of horizontal cells (Hedden and Dowling, 1978; Mangel and Dowling, 1985; Negishi and Drujan, 1979a), alter horizontal cell receptive field size by decreasing electrotonic coupling between cells (Cohen and Dowling, 1983; Gerschenfeld et al., 1982; Lasater and Dowling, 1985; Negishi and Drujan, 1978; Teranishi et al., 1983), and inhibit K^+-stimulated GABA release from horizontal cells (O'Brien and Dowling, 1985; Yazulla and Kleinschmidt, 1982). Since dopamine at physiological concentrations causes neither membrane-potential nor membrane-resistance changes in isolated horizontal cells (Lasater and Dowling, 1985; Lasater et al., 1983), it is believed that the effects of dopamine on horizontal cells are mediated by cAMP (Cohen and Dowling, 1983; Gerschenfeld et al., 1982; Neyton et al., 1982; Teranishi et al., 1983). Although the postsynaptic actions of dopamine are beginning to be understood, very little is known about neural inputs that regulate the synaptic release of dopamine in the teleost retina.

In the goldfish retina, K^+ stimulates a Ca^{2+}-dependent release of ^{3}H-dopamine (Sarthy and Lam, 1979). The indolamine, serotonin (5-HT), has also been reported to stimulate a Ca^{2+}-dependent release of ^{3}H-dopamine in the fish retina (Kato et al., 1982). However, as yet no systematic study of the ability of putative retinal neurotransmitters and neuropeptides to stimulate dopamine release has been reported. Of interest in this regard is the recent finding that the GABA antagonists bicuculline and picrotoxin, like dopamine, alter horizontal cell receptive field size (Negishi et al., 1983; Piccolino et al., 1982). The effects of the GABA antagonists on coupling are blocked by the dopamine antagonist haloperidol and by treatment of the retina with 6-hydroxydopamine (6-OHDA), a neurotoxin that destroys dopaminergic cells (Negishi et al., 1983). These observations suggest the possibility that the GABA antagonists act by releasing dopamine from the retina and that the dopaminergic retinal cells are tonically inhibited by GABA.

GABAergic effects on dopaminergic neurons have previously been described in the rat retina, where muscimol, a GABA agonist, was found to inhibit the light-induced increase in dopamine turnover (Morgan and Kamp, 1980). In addition, GABAergic effects on tyrosine hydroxylase activity in the rat retina have been reported (Marshburn and Iuvonne, 1981). In this paper, we examine the effects of proposed retinal neurotransmitters and neuropeptides, and GABA and glycine antagonists, on ^{3}H-dopamine release from isolated perfused carp (*Cyprinus carpio*) retina. We also describe the effects of the GABA antagonists on cAMP production in the carp retina.

Materials and Methods

Animals

Common carp (*Cyprinus carpio*), 5–7 inches long, were used in all experiments. Animals were maintained on a 12L:12D cycle and dark-adapted for a minimum of 2 hr prior to each experiment.

^{3}H-Dopamine release experiments

Animals were sacrificed, eyes enucleated, and the neural retina dissected free from the retinal pigment epithelium under dim red light. Retinas were placed in oxygenated (95% O_2/5% CO_2) Ringer's containing 0.2 μM ^{3}H-dopamine (31.2 Ci/mmol; New England Nuclear). After a 15 min incubation, retinas were washed in Ringer's for 25 min and thereafter perfused at a rate of 1.4–1.8 ml/min. Retinas were perfused for 20 min prior to the start of an experiment to allow the efflux to reach a stable baseline. Fractions were collected at 1 or 2 min intervals. All fractions were analyzed for ^{3}H content by liquid-scintillation counting. The identity of the ^{3}H label in some samples from drug-stimulated fractions was determined by thin-layer chromatography. Whatman LK5D plates were used with a solvent system containing *N*-butanol/water/acetic acid (12:5:3).

In Table 1, the results are reported as follows: 0, no detectable effect

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Correspondence should be addressed to Patricia O'Connor at the above address.

[1] Present address: Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, England.

Table 1. Effects of neurotransmitters and neuropeptides on the release of tritiated dopamine from carp retina

Compound	Concentration (μM)	Effect on ^{3}H-dopamine release
Amino acids		
Aspartate	10^4, 10^5	0
GABA	10^4, 10^5	0
Glutamate	10^4, 10^5	0
Glycine	10^4, 10^5	0
Peptides		
Enkephalin	10, 10^2	0
Glucagon	10	0
Neuropeptide Y	10^{-1}, 10	0
Somatostatin	10^{-1}, 10	0
Substance P	10^{-1}, 10	0
TRH*	10^3	0
VIP**	10^{-3}, 10^{-1}	0
Other transmitter candidates		
ACh	10^4	0
Carbacol	5×10^2	0
Kainate	10, 10^2	0
Noradrenalin	10, 10^2	0
Quisqualate	10^2, 10^3	0
Serotonin/5-HT	10^2, 5×10^2	+
Tryptamine	10^2, 3×10^2	+
Cyclic AMP related agents		
Forskolin	1, 10^2	0
IBMX	1, 10^3	0
Theophylline	10, 10^2	0
Other		
Amphetamine	10^2, 2×10^2	+
Veratridine	10, 10^2	+

0—no detectable effect.
+—stimulation of ^{3}H release.
−—inhibition of ^{3}H release.
* Thyrotropin releasing hormone.
** Vasoactive intestinal peptide.

on baseline release of dopamine; +, stimulation of ^{3}H-dopamine release; −, depression of ^{3}H-dopamine release. In the dose–response experiments, data are reported as the percentage of total radioactivity released. The percentage released was determined by measuring the radioactivity released during the period of stimulation. The basal release was subtracted from this amount and the resulting amount of radioactivity was divided by the total radioactivity. The total radioactivity was determined by homogenizing the retinas at the end of the experiments and adding to this the amount of radioactivity released during the experiment. In all other experiments, data are reported as counts per minute (cpm).

Autoradiography

Retinas were incubated in ^{3}H-dopamine as described above for 30 min, rinsed for 5 min, and processed for autoradiography according to Yazulla and Kleinschmidt (1982).

cAMP experiments

Retinas (obtained as described above) were placed on filter paper disks and cut into quarter pieces. Each piece was placed in a scintillation vial and 900 μl of Ringer's containing 2 mM isobutylmethylxanthine (IBMX) was added to the vial. Following a 5 min incubation, 100 μl of test substance in Ringer's containing the dopamine uptake inhibitor nomifensine (final concentration 30 μm) was added to the incubation medium and the incubation continued for an additional 10 min (Hunt et al., 1974). For dopamine-blocking experiments, haloperidol was added in the 5 min incubation period prior to the application of the test substance. The reaction was terminated by boiling for 3 min. The incubation Ringer's was removed, and 10 μl samples were assayed in triplicate for cAMP content by the method of Brooker et al. (1979). Protein measurements on the remaining retina pieces were made according to the method of Lowry et al. (1951), using BSA as a standard. cAMP experiments were carried out under dim red light.

Reagents

The Ringer's contained 80 mM NaCl, 22.7 mM $NaHCO_3$, 3.5 mM KCl, 2.4 mM $MgSO_4$, 2.3 mM $CaCl_2$, 10 mM D-glucose, 10 mM HEPES buffer, 0.1 mM ascorbic acid, 15 μm pargyline, pH 7.4. Ca^{2+}-free Ringer's was made up without Ca^{2+} and with 10 mM Mg_2SO_4. Experimental reagents were purchased from the following sources: IBMX (Aldrich), forskolin (CalBiochem), veratridine (ICN Pharmaceutical), neurotensin, neuropeptide Y (Peninsula Labs), glucagon, somatostatin, substance P, thyrotropin-releasing hormone (TRH), and vasoactive intestinal peptide (VIP) (Boehringer Mannheim), bicuculline methochloride (Tocris Chemicals), and haloperidol (McNeil Pharmaceutical). Nomifensine was a generous gift from Hoechst-Roussel Pharmaceutical, Inc. All other reagents were obtained from Sigma.

Results

Uptake of dopamine in the retina, its release, and retinal viability following perfusion

Initially, a number of control experiments were carried out to characterize our methods and the retinal perfusion system. For example, the localization of the ^{3}H-dopamine uptake sites was examined to confirm that dopamine was being taken up by dopaminergic neurons of the retina. Isolated retinas were incubated in ^{3}H-dopamine, rinsed briefly, and then processed for autoradiography. Figure 1 is a light micrograph showing the distribution of ^{3}H-dopamine in the carp retina. The most prominent distribution of grains is found surrounding the horizontal cell bodies, reflecting the dense innervation of the horizontal cells by the dopaminergic interplexiform cells. In addition, there is a labeled cell body in the most proximal layer of the inner plexiform layer. Since the contribution of the dopaminergic interplexiform cell processes to the inner plexiform layer is very small relative to the total area of the inner plexiform layer, it is difficult to detect a significant brain density in the inner plexiform layer over background. From ultrastructural studies, however, it is known that specific uptake of ^{3}H-dopamine does occur in the dopaminergic interplexiform cell process of the inner plexiform layer (Zucker and Yazulla, 1984). The pattern of ^{3}H distribution observed in Figure 1 is similar to that reported by Sarthy and Lam (1979). Also, the identity of the ^{3}H compound released by the retina was determined using thin-layer chromatography. Approximately 70% of the ^{3}H released in the effluent during stimulation with drug colocalized with dopamine. Finally, the viability of the retina following perfusion was assessed by recording the electroretinogram (ERG) at the end of a number of typical experiments. In all cases, both the a- and b-waves of the ERG were within normal limits.

Effect of neurotransmitters and neuropeptides on ^{3}H-dopamine release

For the release studies, retinas were perfused with oxygenated Ringer's until a stable efflux of radioactivity was established. Test substances were introduced into the perfusate for a 3–6 min time period. Table 1 lists the putative retinal neurotransmitters and neuropeptides tested for their ability to affect ^{3}H-dopamine release. The excitatory amino acids glutamate and aspartate, and the glutamate agonists kainate and quisqualate, had no stimulatory effect on ^{3}H-dopamine release. GABA, glycine, ACh, and the ACh agonist carbacol were also without

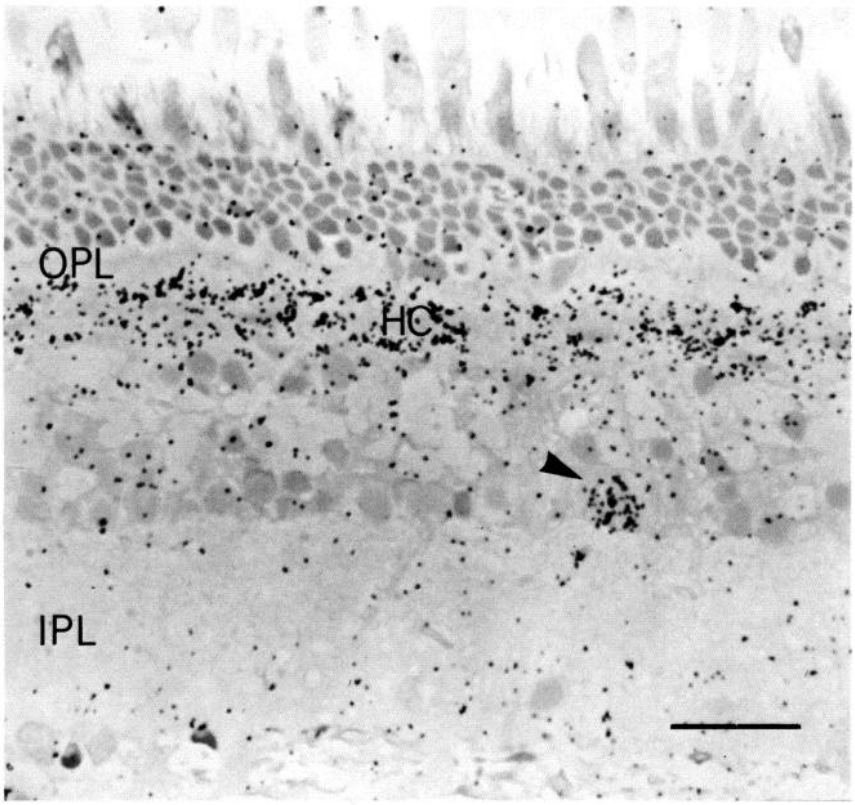


Figure 1. Light micrograph of a 1.5 μm section of carp retina, demonstrating autoradiographic localization of ³H-dopamine. The grain density is most intense in processes surrounding the horizontal cells bodies (*HC*). A labeled interplexiform cell body and some ³H-uptake in the inner plexiform layer (*IPL*) are also visible.

effect. In addition, the neuropeptides enkephalin, glucagon, neuropeptide Y, neurotensin, somatostatin, substance P, TRH, and VIP did not alter ³H-dopamine release from the retina.

The one transmitter candidate that did stimulate ³H-dopamine release was 5-HT. A representative experiment is shown on the left half of Figure 2. An isolated retina was perfused first with normal Ringer's and then with Ringer's containing 500 μM 5-HT for 5 min. A significant increase in ³H-dopamine release was observed in the first minute of application of 5-HT, which was maintained for as long as 5-HT was present in the Ringer's. When normal Ringer's was reintroduced, the efflux of radioactivity returned to the baseline. In experiments such as this one, the stimulatory effect of 5-HT was detectable over the concentration range of 100–500 μM.

The right half of Figure 2 shows, however, that the dopamine release induced by 5-HT was Ca^{2+} independent. Following recovery from the application of 5-HT to the retina, a short pulse of K^+ was applied to the retina, resulting in a sharp increase in dopamine efflux. When Ca^{2+}-free Ringer's was introduced into the perfusion system, K^+ evoked release was inhibited, but the 5-HT evoked release was unaffected.

To test whether 5-HT was releasing dopamine via a receptor-mediated mechanism, the 5-HT antagonist methysergide was used. Retinas were perfused with Ringer's containing 300 μM

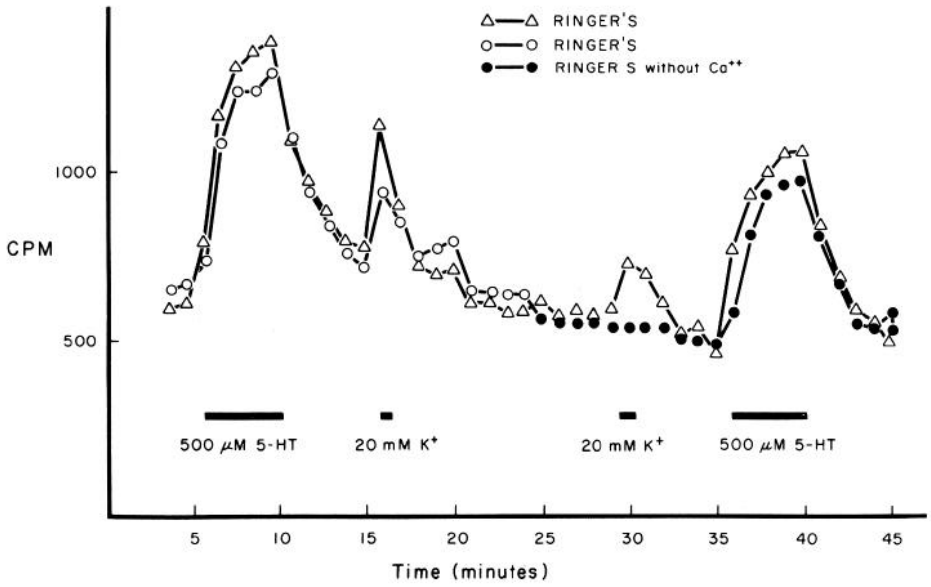


Figure 2. Serotonin-evoked ³H-dopamine release in normal and Ca^{2+}-free Ringer's. Two retinas from the same fish are represented above. One retina received 2 pulses of 20 mM K^+ and 500 μM 5-HT in normal Ringer's (*open triangles*). The other retina received a single pulse of 20 mM K^+ and 500 μM 5-HT in normal Ringer's (*open circles*), followed by identical pulses in Ca^{2+}-free Ringer's (*closed circles*). Although K^+ failed to evoke release in Ca^{2+}-free Ringer's, 5-HT evoked release in Ca^{2+}-free Ringer's was almost identical to 5-HT evoked release in normal Ringer's.

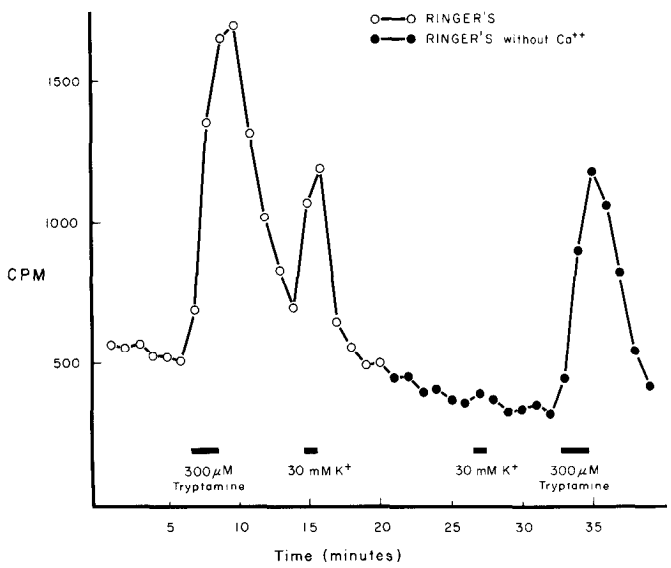


Figure 3. Tryptamine-evoked ^{3}H-dopamine release in normal and Ca^{2+}-free Ringer's. Representative tracing from an experiment that demonstrates the Ca^{2+} independence of tryptamine-evoked release of ^{3}H-dopamine. When retina was perfused in normal Ringer's (*open circles*), both 300 μM tryptamine and 30 mM K^+ evoked release of ^{3}H-dopamine. In Ca^{2+}-free Ringer's (*closed circles*), 30 mM K^+ failed to evoke ^{3}H-dopamine release, whereas tryptamine-evoked release was unaffected.

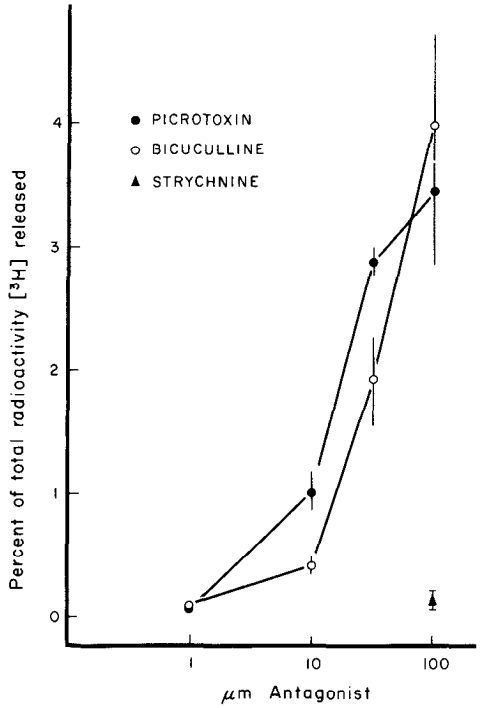


Figure 4. Dose-dependent effects of bicuculline and picrotoxin on ^{3}H-dopamine release. Retinas were perfused in normal Ringer's followed by a 6 min perfusion in Ringer's containing the test substance. Results were calculated as the percentage of total tissue radioactivity released above baseline. Each point is the mean of at least 2 experiments.

methysergide and with Ringer's containing both methysergide and 5-HT. Methysergide failed to inhibit significantly the 5-HT evoked release of dopamine. Tryptamine, a 5-HT agonist, also stimulated release of ^{3}H-dopamine but, as was the case with 5-HT, the release evoked by tryptamine was Ca^{2+} independent (Fig. 3).

We also examined the effects of agents known to elevate cAMP levels for their ability to affect dopamine release. Forskolin, IBMX, and theophylline, at concentrations that elevate cAMP in the retina, were all ineffective (Table 1). In addition, we tested the ability of amphetamine and veratridine to release dopamine. Amphetamine induced release of ^{3}H-dopamine, which is consistent with the known actions of this drug in facilitating dopamine release in the CNS (Arnold et al., 1977; Horn et al., 1974; McKenzie and Szerb, 1968). The depolarizing agent veratridine also stimulated a significant release of ^{3}H-dopamine.

Effect of GABA antagonists on ^{3}H-dopamine release

Both bicuculline and picrotoxin stimulated a dose-dependent release of ^{3}H-dopamine (Fig. 4). In these experiments, retinas were perfused with normal Ringer's followed by a 6 min perfusion with either bicuculline or picrotoxin. The release is expressed as the percentage of total tissue radioactivity. Increasing concentrations of the GABA antagonists caused a graded release of ^{3}H-dopamine. The concentrations of the GABA antagonists employed in our experiments were the same concentrations that have been reported to uncouple horizontal cells (Negishi et al., 1983; Piccolino et al., 1982). The effects of the GABA antagonists appeared selective, as 100 μM strychnine, a glycine antagonist, failed to induce dopamine release (Fig. 4).

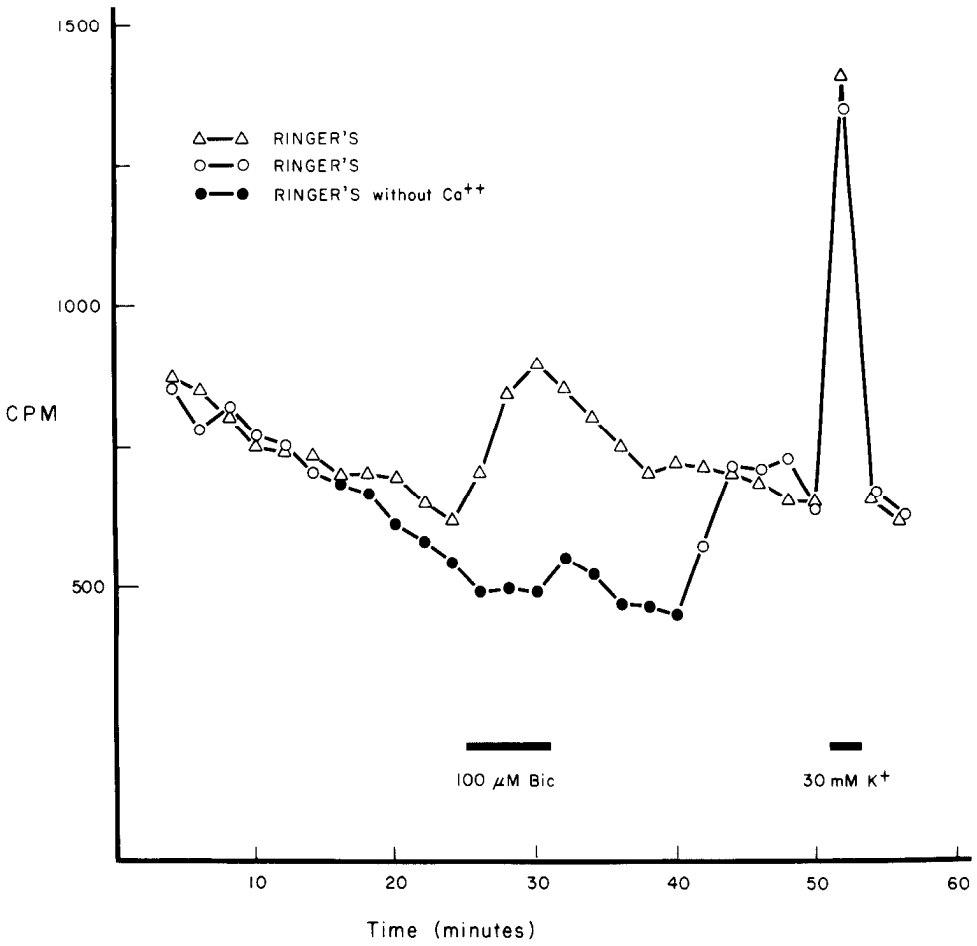
The release evoked by bicuculline and picrotoxin was Ca^{2+} dependent. Neither of these agents caused a significant release of ^{3}H-dopamine when applied in Ca^{2+}-free Ringer's (Fig. 5). When Ca^{2+}-free Ringer's was replaced with normal Ringer's, there was an immediate increase in dopamine efflux. Following this, a typical release of ^{3}H-dopamine could be evoked by 30 mM K^+ (Fig. 5). In several experiments we tried to elucidate the basis for the sharp increase in dopamine release following the substitution of normal Ringer's. When retinas were perfused with Ca^{2+}-free Ringer's in the absence of drug, the "rebound" effect still occurred. This ruled out the possibility that residual drug was bound to the receptor, suggesting the possibility that altering ionic conditions (i.e., Mg^{2+}, Ca^{2+}) causes membrane potential changes sufficient to release dopamine.

Finally, to determine the specificity of the GABA antagonist effect on dopamine release, retinas were perfused with 5 mM GABA prior to the application of bicuculline and picrotoxin. GABA completely blocked the release of ^{3}H-dopamine evoked by bicuculline and significantly reduced the release evoked by picrotoxin (Fig. 6).

Effect of GABA antagonists on dopamine-sensitive adenylate cyclase

Our findings that the GABA antagonists release dopamine, coupled with previous findings of a dopamine-sensitive adenylate cyclase in the retina, suggested that these agents might also alter cAMP levels. In initial experiments, we observed a small (40–50 pmol cAMP/mg protein) increase in cAMP accumulation above control levels when 100 μM bicuculline or picrotoxin was applied to the retina (Fig. 7). In subsequent experiments, nomifensine, a dopamine uptake inhibitor (Hunt et al., 1974), was added to the incubation medium and was found to enhance significantly the accumulation of cAMP signal. In the presence of 30 μM nomifensine, the GABA antagonists (100 μM) stimulated an approximate 300 pmol cAMP/mg protein increase above control levels (Fig. 7). Strychnine, which does not release ^{3}H-dopamine, did not stimulate cAMP accumulation over basal levels. We also observed that nomifensine significantly increased basal levels of cAMP, by about 180 pmol/mg protein (Fig. 7).

To show that the GABA antagonists increased cAMP accumulation by releasing dopamine, the effects of bicuculline and picrotoxin were tested in the presence of the dopamine antag-



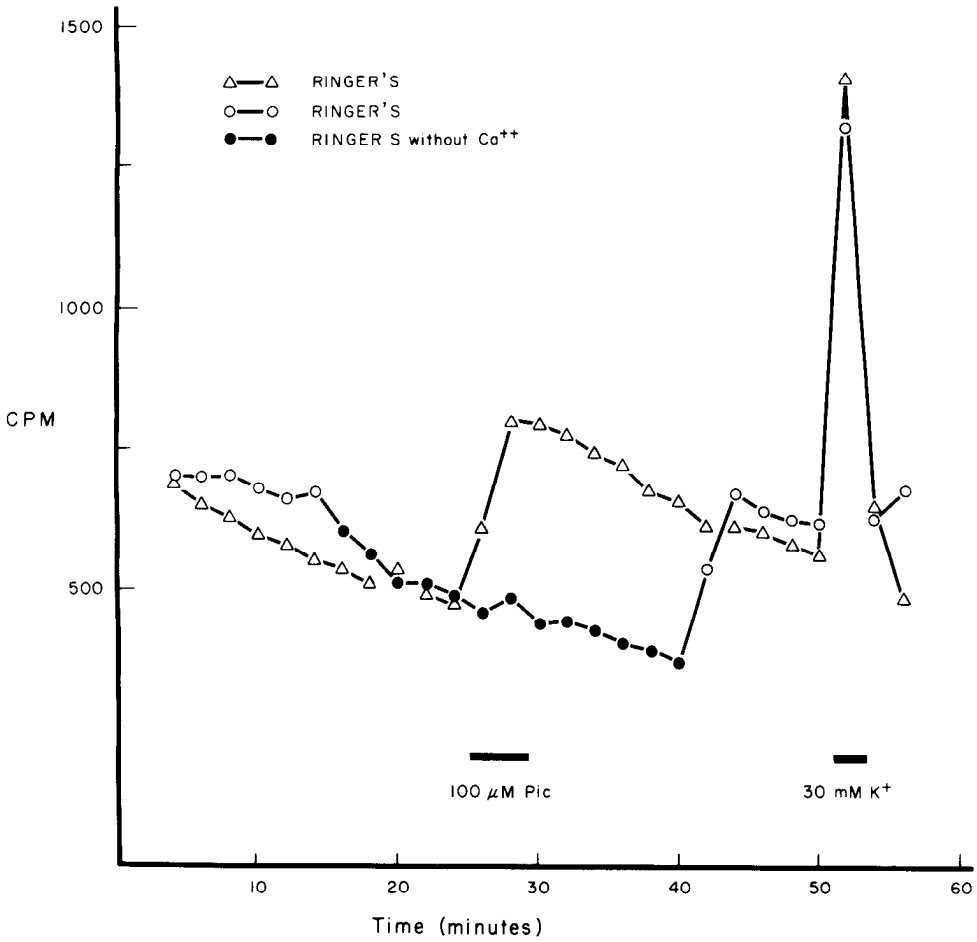
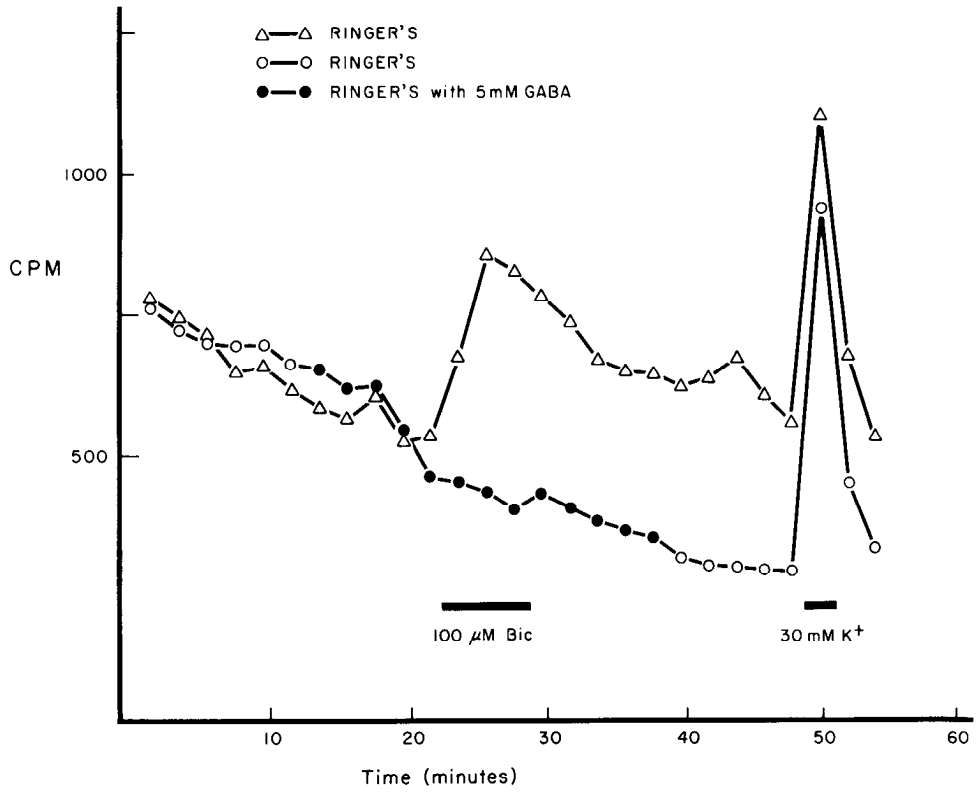


Figure 5. Effects of Ca^{2+}-free Ringer's on bicuculline- (*top*) and picrotoxin- (*bottom*) stimulated release of ^{3}H-dopamine. One retina was perfused continuously with normal Ringer's (*open triangles*). Both bicuculline and picrotoxin evoked release of ^{3}H-dopamine. K^+, 30 mM, also released dopamine. The contralateral retina was perfused with normal Ringer's (*open circles*) and then with Ca^{2+}-free Ringer's (*closed circles*). Neither bicuculline nor picrotoxin evoked release of ^{3}H-dopamine in Ca^{2+}-free Ringer's. When Ca^{2+}-free Ringer's was replaced with normal Ringer's (*open circles*), a typical release of ^{3}H-dopamine was evoked by 30 mM K^+. Each point represents data averaged from 4 experiments. The SEM is less than 15% of the mean unless otherwise indicated.



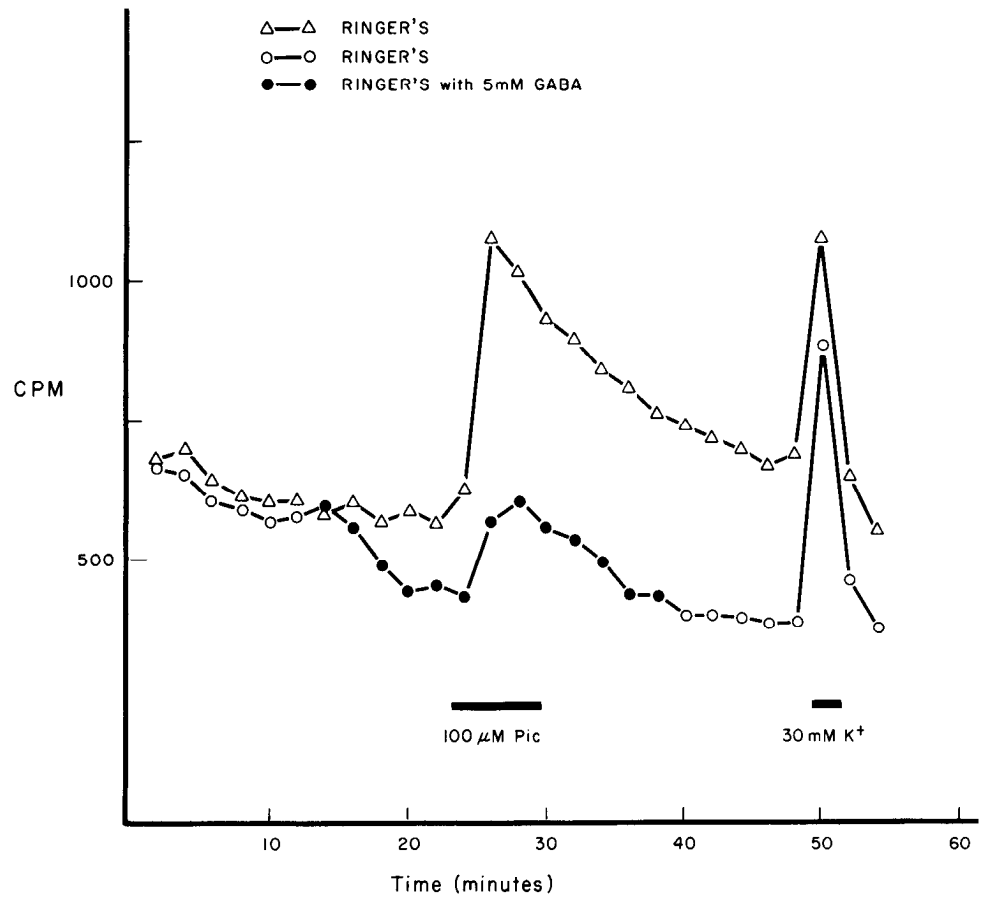


Figure 6. Effect of GABA on bicuculline- (*top*) and picrotoxin- (*bottom*) stimulated release of ^{3}H-dopamine. One retina was perfused continuously with normal Ringer's (*open triangles*). Both bicuculline and picrotoxin evoked release of ^{3}H-dopamine. K^+, 30 mM, also released dopamine. The contralateral retina was perfused with normal Ringer's (*open circles*) and then with Ringer's containing 5 mM GABA (*closed circles*). Perfusion with 5 mM GABA completely blocked the release evoked by bicuculline and significantly blocked the release evoked by picrotoxin. When normal Ringer's was reintroduced (*open circles*), a typical release of ^{3}H-dopamine was evoked by 30 mM K^+. Each point represents data averaged from 4 experiments. The SEM is less than 15% of the mean unless otherwise indicated.

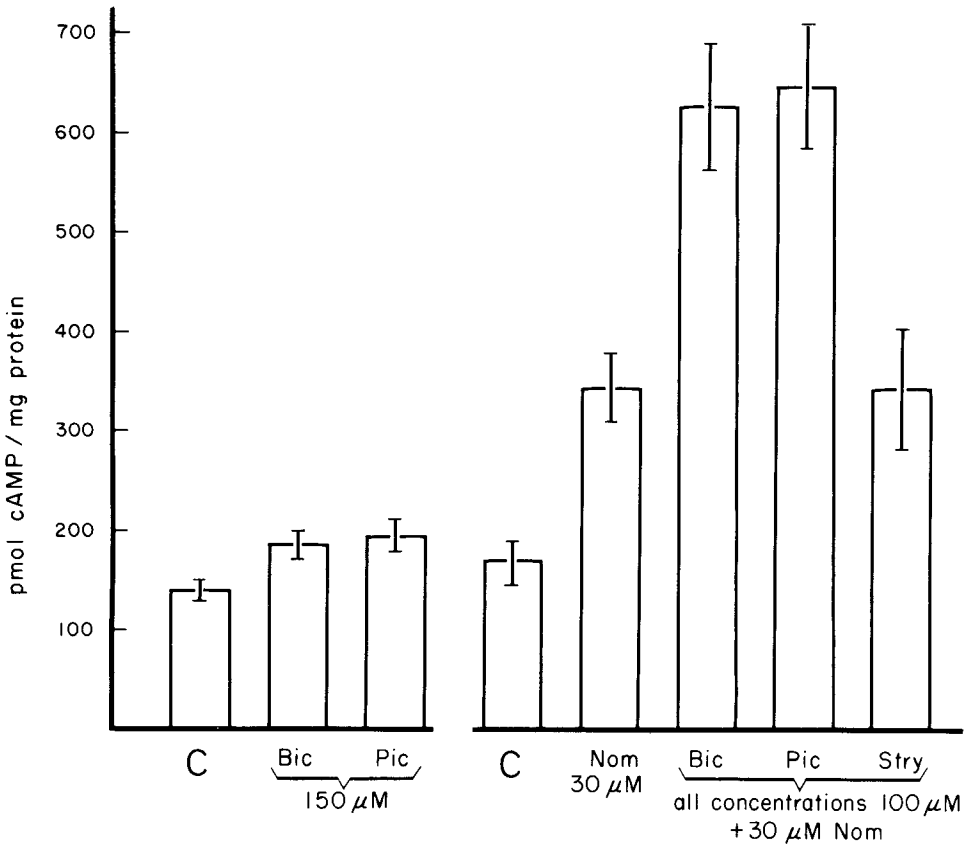


Figure 7. Effect of GABA and glycine antagonists on cAMP levels in intact carp retina. In the absence of the dopamine-uptake inhibitor nomifensine, bicuculline and picrotoxin stimulated an approximate 50 pmol cAMP/mg protein increase over basal levels. When nomifensine was added to the incubation medium, the GABA antagonists stimulated a 300 pmol cAMP/mg protein increase. Strychnine, which does not release ³H-dopamine, did not affect cAMP levels. Values represent the mean ± SEM of at least 4 experiments.

onist haloperidol. When haloperidol was added to the incubation medium, the stimulation of cAMP accumulation induced by 100 μM bicuculline or picrotoxin was completely blocked (Fig. 8). Figure 8 also shows that haloperidol alone depressed basal levels of cAMP.

Discussion

In this study, an isolated perfused retinal preparation was used to examine the ability of putative retinal neurotransmitters and neuropeptides to affect release of ³H-dopamine from carp retina. Of the transmitter candidates tested (Table 1), only 5-HT and the 5-HT agonist, tryptamine, stimulated ³H-dopamine release from the retina. The release evoked by these agents was Ca^{2+} independent and, hence, probably not synaptic. A similar observation has been reported in rabbit retina (Dubocovich, 1983). Concentrations of 5-HT higher than 1 μM significantly increased basal efflux of ³H through a Ca^{2+}-independent process. Our findings disagree with those of a previous report by Kato et al. (1982), which showed that 5-HT stimulated a Ca^{2+}-dependent release of ³H-dopamine from a particulate fraction of carp retina. Since perfused intact retinas were used in our experiments, we think that our results more closely reflect the *in vivo* situation.

One possible explanation for our data is that 5-HT causes cytoplasmic dopamine release via an exchange mechanism. That is, exogenous 5-HT is taken up by the dopaminergic cells, causing a displacement and release of endogenous dopamine. Several observations support this hypothesis. First, it is known that the dopaminergic cells of the carp retina are capable of accumulating 5,6-dihydroxytryptamine, an indoleamine (5-HT) analog (Kato et al., 1982). Second, the Ca^{2+} independence of 5-HT induced dopamine release is consistent with a cytoplasmic exchange mechanism. Third, an exchange mechanism does not require receptor activation. In agreement with Kato et al. (1982), we have found that the 5-HT antagonist methysergide does not block the 5-HT evoked release of ³H-dopamine.

A surprising finding was the inability of other tested retinal transmitter or peptide candidates to affect release of dopamine. Previous studies had suggested that ACh and substance P might release dopamine in the fish retina (Negishi and Drujan, 1979b; Negishi et al., 1982). For example, both ACh and substance P appeared to diminish the catecholamine histofluorescence in retinas preloaded with dopamine (Negishi et al., 1980), and it was reported that high, millimolar concentrations of ACh induce effects on the receptive field properties of L-type horizontal cells similar to those of dopamine (Negishi and Drujan, 1979a, b). In our experiments, we directly measured the release of ³H-dopamine from the retina and were unable to detect any effect of ACh or substance P on dopamine release. We can rule out the loss of retinal viability as a reason for the failure of the various agents to be active, since normal ERGs were recorded from retinas after typical perfusion experiments. Also, relatively low concentrations of the GABA antagonists stimulated release of ³H-dopamine. It may be that there exists an unidentified excitatory synaptic input to the dopaminergic cells, but another possibility is that there is no excitatory input to these cells. Rather, the interplexiform cell at rest may release dopamine, and this release may be controlled by modulation of an inhibitory input.

Our results clearly indicate that a GABAergic inhibitory input is important in regulating dopamine release in the carp retina. Both bicuculline and picrotoxin released ³H-dopamine from the retina and activated a dopamine-sensitive adenylate cyclase as a consequence of their ability to release dopamine. As in the case of K^+-stimulated dopamine release, the release evoked by these agents was Ca^{2+} dependent, indicative of a synaptic mechanism (Katz and Miledi, 1967). It is likely that the GABA antagonists induce dopamine release by specifically blocking the actions of endogenous GABA. The concentrations of bicuculline and picrotoxin employed in our experiments were sufficiently low, so it is unlikely that their effects were due to nonspecific

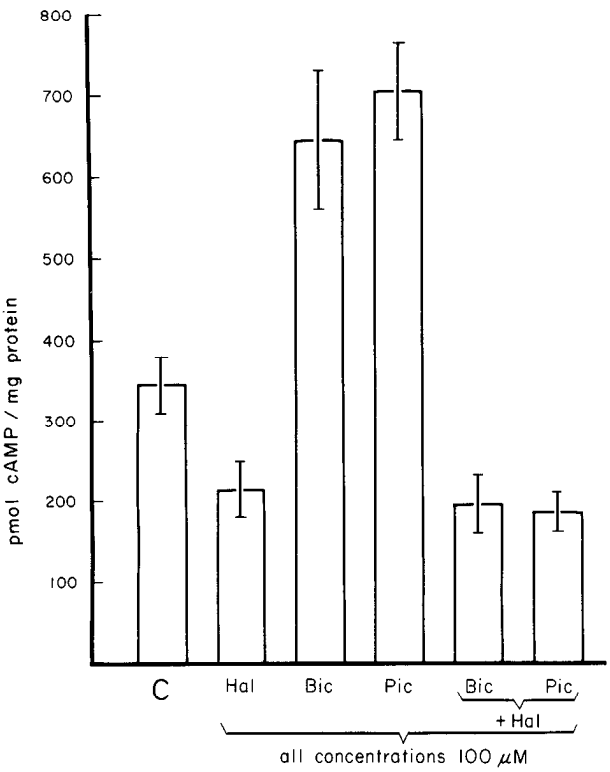


Figure 8. Effect of haloperidol on GABA antagonist-stimulated cAMP accumulation. Incubation of the retinas with 100 μM haloperidol completely blocked the effect of the GABA antagonists on cAMP accumulation. Values represent the mean ± SEM of at least 4 experiments.

actions. In this regard, Miller et al. (1981b) reported that bicuculline and picrotoxin acted specifically at 10^{-4} M or less in the mudpuppy retina. Also, release was not stimulated by equivalent concentrations of the glycine antagonist strychnine. Finally, perfusion with exogenous GABA completely blocked the bicuculline- and picrotoxin-evoked release. The finding that GABA did not completely block the picrotoxin effect might be attributed to the fact that GABA and bicuculline are thought to bind to a different site than picrotoxin (Andrews and Johnston, 1979). In a receptor-binding study, it was reported that bicuculline, but not dihydroxypicrotoxin, displaced ^{3}H-GABA (Olsen et al., 1978). Hence, the noncompetitive interactions of GABA and picrotoxin could explain the lack of total inhibition. A similar finding in rabbit retina has been reported in which muscimol inhibits bicuculline actions but fails to inhibit picrotoxin (Massey and Redburn, 1982).

In addition to their effects on dopamine release, bicuculline and picrotoxin also stimulated a dopamine-dependent cAMP accumulation. The increase in cAMP was blockable by haloperidol, indicating these agents release endogenous dopamine that exerts the same postsynaptic actions as exogenously applied dopamine. For these experiments, we used the dopamine uptake inhibitor nomifensine to optimize cAMP accumulation. In the presence of nomifensine, we observed about a 300 pmol increase in cAMP accumulation in response to 100 μM bicuculline or picrotoxin, as compared to a 50 pmol elevation of cAMP in the absence of nomifensine. The enhanced effects observed in the presence of nomifensine clearly demonstrate the effectiveness of uptake systems in regulating synaptic actions of dopamine in the retina.

Our findings provide direct evidence that the dopaminergic interplexiform cells of the teleost retina are under a tonic inhibitory control by GABAergic neurons. Based on our cAMP experiments, it appears that the GABAergic input does not totally inhibit the dopaminergic cell. Nomifensine causes an elevation of basal cAMP levels, while haloperidol depresses basal cAMP levels. Taken together, these observations indicate that a dopamine release occurs in the basal state under our experimental conditions. This basal release of dopamine can be increased by the GABA antagonists bicuculline and picrotoxin. For reasons that are not clear, we have not consistently observed a depression of ^{3}H-dopamine release by GABA (but see Fig. 6, bottom). On the other hand, we have consistently observed a significant depression of basal cAMP levels by the addition of GABA to the retina.

Our findings of an ongoing release of dopamine agree with the recent evidence (Mangel and Dowling, 1985) that there is a release of dopamine in the retina, presumably from interplexiform cells following a prolonged period in the dark. They reported that 100–110 min of complete darkness reduced the responsiveness and receptive field size of cone horizontal cells, as does dopamine application. Since we routinely dark-adapted our fish for 2–4 hr prior to an experiment, it is expected that a substantial basal release of dopamine should be detected in our cAMP experiments.

The concept of tonic GABA release and modulation of GABAergic activity as a mechanism for regulating transmitter release in the retina has been proposed earlier (Massey and Redburn, 1982; Miller et al., 1981a; Neal and Massey, 1980). In the rabbit retina, bicuculline and picrotoxin enhance basal efflux as well as light-evoked release of ^{3}H-ACh, whereas GABA and muscimol depress release of ^{3}H-ACh (Massey and Redburn, 1982; Neal and Massey, 1980). A tonic release of GABA has also been hypothesized in mudpuppy retina (Miller et al., 1981a). Thus, in addition to an excitatory input stimulating transmitter release, it seems likely that suppression of an inhibitory input can also release transmitter. Whether disinhibition of GABA input is the only mechanism for regulating dopamine release in the retina is as yet unclear.

Localization of GABAergic and dopaminergic cells in the teleost retina is consistent with the possibility of interactions between these 2 transmitter systems. A recent study on the synaptic connections of the dopaminergic interplexiform cell in the goldfish retina reported that the majority of inputs are from processes found within the most proximal and distal laminae of the inner plexiform layer (Zucker and Yazulla, 1984). Hence, it is possible that the interplexiform cell receives input from a class of GABAergic neurons, the modulation of which would serve to regulate dopamine release.

Our results are also consistent with previous suggestions (Negishi et al., 1983) that the GABA antagonists affect horizontal cell receptive field size by acting on the dopaminergic interplexiform cells, causing the release of dopamine and a consequent activation of the dopamine-sensitive adenylate cyclase of the horizontal cell. Although the exact cellular location of the cAMP increase has not been shown, the horizontal cell is the most likely candidate. From anatomical studies, it is known that the interplexiform cells make abundant synapses on the horizontal cells (Dowling and Ehinger, 1978). In addition, a potent dopamine-sensitive adenylate cyclase has been localized to isolated horizontal cells (Van Buskirk and Dowling, 1981). Furthermore, we have evidence that ^{3}H-dopamine release occurs from the interplexiform cell processes surrounding the horizontal cell bodies in the outer plexiform layer (P. O'Connor, C. Zucker, and J. E. Dowling, unpublished observations). That is, using LM autoradiography, we have labeled dopamine uptake sites in the carp retina and have found that when retinas are treated with 100 μM bicuculline or picrotoxin, there is a significant decrease in grain density from labeled processes in the outer plexiform layer.

Finally, our studies and those of others (Gerschenfeld et al.,

1982; Negishi et al., 1983) indicate that caution should be exercised when analyzing pharmacological experiments carried out on intact pieces of tissue. Effects that are observed when using specific antagonists may result not simply from blockade of postsynaptic receptors. For example, it is clear from our data that the effect of the GABA antagonists on cAMP accumulation in the carp retina results from the release of dopamine evoked by these drugs. Thus, the actions of the GABA antagonists in reducing junctional conductance of the retinal horizontal cells (Negishi et al., 1983; Piccolino et al., 1982) are also likely to result from the release of dopamine in the retina rather than as a direct effect of these agents on the horizontal cells. It is also known that GABA antagonists release ³H-ACh in rabbit retina (Massey and Redburn, 1982; Neal and Massey, 1980). Thus, in studies using GABA antagonists, it is essential that the effects of these drugs on transmitter release from presynaptic terminals be considered in the interpretation of experimental results.

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