Adenosine A1 and Class II Metabotropic Glutamate Receptors Mediate Shared Presynaptic Inhibition of Retinotectal Transmission

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Zhang, Chunyi and John T. Schmidt. Adenosine A1 and class II metabotropic glutamate receptors mediate shared presynaptic inhibition of retinotectal transmission. J. Neurophysiol. 82: 2947–2955, 1999. Presynaptic inhibition is one of the major control mechanisms in the CNS. Previously we reported that adenosine A1 receptors mediate presynaptic inhibition at the retinotectal synapse of goldfish. Here we extend these findings to metabotropic glutamate receptors (mGluRs) and report that presynaptic inhibition produced by both A1 adenosine receptors and group II mGluRs is due to G\textsubscript{i} protein coupling to inhibition of N-type calcium channels in the retinal ganglion cells. Adenosine (100 \textmu M) and an A1 (but not A2) receptor agonist reduced calcium current (I_{Ca,\text{L}}) by 16–19\% in cultured retinal ganglion cells, consistent with their inhibition of retinotectal synaptic transmission (30\% amplitude of field potentials). The general metabotropic glutamate receptor (mGluR) agonist 1S,3R-1-amino-cyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD, 50 \textmu M) and the selective group II mGluR receptor agonist (2S,2',3,3'R)-2-(2',3',3'-dicarboxy-cyclopropyl)glycine (DCG-IV, 300 nM) inhibited both synaptic transmission and I_{Ca,\text{L}} whereas the group III mGluR agonist t-2-amino-4-phosphono-butrate (t-AP4) inhibited neither synaptic transmission nor I_{Ca,\text{L}}. When the N-type calcium channels were blocked with \alpha-conotoxin GVIA, both adenosine and DCG-IV had much smaller percentage effects on the residual 20\% of I_{Ca,\text{L}}, suggesting effects mainly on the N-type calcium channels. The inhibitory effects of A1 adenosine receptors and mGluRs were both blocked by pertussis toxin, indicating that they are mediated by either G\textsubscript{i} or G\textsubscript{j}. They were also inhibited by activation of protein kinase C (PKC), which is known to phosphorylate and inhibit G\textsubscript{i}. Finally, when applied sequentially, inhibition by adenosine and DCG-IV were not additive but occluded each other. Together these results suggest that adenosine A1 receptors and group II mGluRs mediate presynaptic inhibition of retinotectal synaptic transmission by sharing a pertussis toxin (PTX)-sensitive, PKC-regulated G\textsubscript{i} protein coupled to N-type calcium channels.

INTRODUCTION

Synaptic transmission is strongly modulated by neurotransmitter control of the calcium channels mediating vesicle release (Sanchez-Prieto et al. 1996). Often these receptors furnish autoregulation of release by responding to transmitters released from the same terminal, such as glutamate and adenosine, and by coupling directly via G-proteins to inhibition of calcium channels (Chavis et al. 1994; Clapham 1994; Swartz and Bean 1992; Trombley and Westbrook 1992; Yawo and Chuhma 1993; Zhou et al. 1995). These modulations by adenosine and metabotropic glutamate receptors are of interest not only because of their ability for fast, second-to-second reshaping of transmission (Swartz et al. 1993), but also because of their implication in controlling synaptic plasticity (Pekhletska 1996) and in controlling neurodegeneration caused by excess glutamate release (Nicoletti et al. 1996). In the visual system, the retinal ganglion cells contain adenosine and have adenosine A1 (but not A2) receptors on their terminals in optic tectum (Blazynski et al. 1989; Braas et al. 1987; Goodman et al. 1983; Wan and Geiger 1990). In a previous study, we showed that retinotectal synaptic transmission in goldfish is mainly mediated by N-type calcium channels and is inhibited by activation of presynaptic adenosine A1 receptors (Zhang and Schmidt 1998). Blocking A1 receptors increased synaptic transmission, indicating the presence of tonic adenosine inhibition. The inhibition was mediated by pertussis toxin (PTX)-sensitive G-proteins and was interrupted by C-kinase activation. However, there have been no systematic studies addressing the effect of adenosine receptors on calcium channels in this synapse.

The presynaptic effects of metabotropic glutamate receptors (mGluRs) in the visual system have also not been thoroughly explored in spite of the potential effects on the transmission of visual information and on visual plasticity. In hippocampus (Baskys and Malenka 1991; Desai et al. 1994), cerebellum (Chavis et al. 1994), cortex (Burke and Hablitz 1994), olfactory bulb (Trombley and Westbrook 1992), and striatum (East et al. 1995; Swartz et al. 1993), activation of either class II or class III metabotropic receptors presynaptically modulates glutamate release, giving rise to an autoregulation of release. The retinotectal projection is also known to use glutamate as its transmitter (Kageyama and Meyer 1989; Langdon and Freeman 1987; Schmidt 1991). Although one paper (Rothe et al. 1994) examined the effect of metabotropic receptor activation on calcium channels in ganglion cells, the authors used a nonselective agonist that did not distinguish which class of metabotropic receptors produced inhibition and which produced augmentation of the calcium currents; nor did they relate the effects to presynaptic inhibition.

In the present study, we use both the isolated nerve-tectum preparation and cultured goldfish retinal ganglion cells for parallel studies on presynaptic inhibition and calcium channel modulation, and report that activation of A1 adenosine receptors and group II metabotropic glutamate receptors both inhibit N-type calcium channels to produce the presynaptic inhibition. The parallel effects of adenosine A1 and class II metabotropic receptors also prompted us to investigate whether they shared...
a common mechanism. Both were sensitive to activation of protein kinase C (PKC) and to PTX treatment, indicating coupling via the same G
\textsubscript{i} protein to the N-type calcium channels. Moreover, inhibition by one occluded the inhibition by the other, verifying that some part of the mechanism is common to both.

**METHODOLOGY**

**Field potential recordings**

The recording of extracellular synaptic potentials elicited by electrical stimulation of optic nerve are described in detail in the previous paper (Zhang and Schmidt 1998). The dissection of the in vitro nerve-tectum preparation, the flow chamber, and the electronic equipment were exactly as in the previous report.

**Cell culture**

Calcium current recording experiments were performed on cultured retinal ganglion cells of goldfish (*Carassius auratus*). The general cell-culture procedures have been previously reported (Schmidt et al. 1991). Briefly, the optic nerves of goldfish were crushed 10 days before cell culture to facilitate axonal regeneration. After dark adaptation, the eyes were removed and the retinas dissected out. The retina was blotted on filter paper to remove the photoreceptor layer, then treated with bacterial neutral protease (Sigma, 0.4 mg/ml) for 30 min while bubbling with O\textsubscript{2}. After washing three times with cold dissection buffer containing 1% BSA, trypsin protease inhibitor, and kynurenate, the retinas were triturated in culture medium with DNAse (0.25 mg/ml) and protease inhibitor (15 μg/ml). The cell suspension was distributed to coverslips coated with laminin (25 μg/ml) in 35-mm culture dishes. The L15 culture medium was supplemented with 10% fetal calf serum and 0.1 mg/ml gentomycin (all from Sigma). Controlled cultures were generally recorded during the first 7–10 days.

**Recording of membrane calcium currents**

Calcium currents were recorded from ganglion cells using the whole cell patch-clamp technique. The current signal was amplified with PC-One Patch Clamp Amplifier (Dagan Corporation, Minneapolis, MN) and was digitized and stored in a 386 computer with Data Translation 2801A A/D board and software written by John Dempster (available from Dagan). The ganglion cells were identified by their slightly oval shape, relatively large nuclei, and conspicuous nucleoli (Ishida and Cohen 1988). In 1-wk-old cultures, the ganglion cells usually were 12–18 μm diam and had a long axon extending from one pole of the multipolar cell body.

The culture dish was used as a recording chamber. Cells were continuously superfused with a solution containing the following (in mM): 88 NaCl, 30 TEA-Cl, 10 glucose, 22.5 HEPES, 1 MgCl\textsubscript{2}, 2.7 KCl, and 5 CaCl\textsubscript{2}. The solution was oxygenated by bubbling with pure O\textsubscript{2}. The superfusion was gravity driven at a flow rate of 1 ml/min. In all experiments where calcium current was recorded, tetrodotoxin (0.5 μM, Sigma) blocked the sodium currents, and tetraethylammonium (TEA) inhibited potassium currents. Ionic currents were recorded with glass microelectrodes pulled from 1.5-mm glass capillaries (TW150F-4, World Precision Instruments, Sarasota, FL), and fire-polished on a heated filament. The pipette solution contained the following (in mM): 40 CsCl, 10 TEA-Cl, 10 EGTA, 10 glucose, 25 HEPES, 1 MgCl\textsubscript{2}, 0.5 CaCl\textsubscript{2}, 2 ATP, 0.1 GTP, and 20 creatine phosphate with 50 U/ml creatine phosphokinase. The DC resistance of the pipettes was 4–6 MΩ in bath solution. The low free calcium in this pipette solution (estimated at 10 nM) was to prevent calcium-driven rundown of the calcium current or contamination by Ca\textsuperscript{2+}-dependent K\textsuperscript{+} or Cl\textsuperscript{−} currents. After obtaining a gigahm seal, we compensated for series resistance (7–10 MΩ) and electrical capacitance. Unless otherwise stated, the holding potential was set at −90 mV. Membrane calcium current was evoked by applying a depolarizing pulse of 90 mV × 100 ms, except in experiments where the current-voltage relationship was studied. The rundown of calcium current, monitored in preliminary experiments, averaged <3% per hour (n = 5), a much longer period of time than that required for testing the effect of a drug on calcium current. Thus rundown was not a problem under the present recording conditions.

For purposes of testing for adenosine- and mGluR-mediated effects on input resistance and membrane potential, we replaced the external TEA with NaCl and omitted TTX, and we used an intracellular solution based on KMeSO\textsubscript{4} replacement of CsCl and TEA-Cl, with other components the same. This is labeled “normal” solution conditions to distinguish from solutions (above) that isolate calcium currents. The cells were recorded in current clamp with the whole cell patch technique and tested with −50 pA × 100 ms current steps to determine input resistance in the presence or absence of adenosine and (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV).

**Application of drugs by superfusion**

A SF-77B Perfusion Fast-Step (Warner Instrument, Hamden, CT) was used to switch between different perfusion solutions within a second. For concentration-response curves, the drug was started at its lowest concentration and switched to the next higher concentration when the preceding concentration reached its plateau effect, which occurred in <2 min.

**Data analysis**

Numerical data reported in the text are means ± SE. Groups of 10 current traces were collected, leak subtracted, and averaged with the computer program for each measurement; 2–3 such measurements were made before drug application, at the plateau of drug action and after drug wash out. Values measured after wash out were averaged with the predrug values for use as a control. Comparison of means was performed with either Student’s t-test or one-way ANOVA as appropriate. A P value <0.05 was regarded as statistically significant.

**Drugs used**

Adenosine and tetradecanoyl phospholipid (TPA) were obtained from Sigma. Cyclohexyladenosine (CHA) and N\textsuperscript{6}-(2-[3,5-dimethoxyphenyl]-2-(2methylphenyl)-ethyl) adenine (DPMA) were obtained from Research Biochemicals (Natick, MA). t-Amino-4-phosphonobutyrate (t-AP4), 1S,3R-1-amino-1-cyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD), and (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV) were obtained from Tocris Cookson (Ballwin, MO). Lactam, TTX, and α-conotoxin GVIA (α-Ctx GVIA) were obtained from Calbiochem (La Jolla, CA).

**RESULTS**

**Inhibitory effect of adenosine A1 receptors**

We previously reported that adenosine presynaptically inhibits retinotectal synaptic transmission by acting on A1 adenosine receptors (Zhang and Schmidt 1998). The effect was presynaptic as judged by increased paired-pulse facilitation. Examples of this inhibition are shown in Fig. 1A: the field potential elicited by stimulation of the optic nerve and recorded in the retinal terminal layer of goldfish tectum was inhibited by adenosine and by the selective A1 receptor agonist CHA but not by the selective A2 receptor agonist DPMA. In addition, the adenosine inhibition was fully reversed by the selective A1
antagonist 8-cyclopentyl-1,3-dipropylxanthine. These results suggested that this effect was due to a G-protein–mediated inhibition of N-type calcium channels controlling vesicle release from retinal terminals (Zhang and Schmidt 1998).

Although it is not possible to record calcium currents from retinal terminals, we could use the whole cell patch technique to record them from retinal ganglion cells in cultures. First, to rule out the possibility that adenosine activation of K channels (Trussel and Jackson 1987) could mediate the presynaptic inhibition, we recorded retinal ganglion cells in voltage clamp under conditions that isolate calcium currents. Consistent with the presynaptic inhibition results above, the calcium currents recorded in retinal ganglion cells were significantly inhibited by adenosine and CHA and the lack of inhibition by DPMA. B: whole cell patch recordings of cultured ganglion cells in which calcium currents were isolated as described in METHODS. Membrane potential was held at −90 mV, and calcium current was evoked by a depolarizing pulse of 100 ms duration and 90 mV amplitude. Note that the calcium current was significantly decreased by adenosine (100 μM, a) and CHA (100 nM, b) but not by DPMA (100 nM, c).

In parallel to the adenosine study above, we tested the effects of activating metabotropic glutamate receptors (mGluRs) both on retinotectal synaptic transmission and on calcium currents in ganglion cells. Metabotropic glutamate receptors are classified into several groups based on pharmacological profiles and on molecular cloning (Abe et al. 1992; Okamoto et al. 1994; Saugstad et al. 1994; see also review by Pin and Duvoisin 1995).

In the present study of presynaptic inhibition, we tested the general mGluR agonist 1S,3R-ACPD, the group II mGluR agonist DCG-IV, and the group III agonist L-AP4, all of which have been linked to presynaptic inhibition in other systems. As shown in Fig. 3A, the general agonist, 1S,3R-ACPD (50 μM), which is somewhat more active at group II mGluRs than at other mGluRs, inhibited the field potential by 17.90 ± 1.97% (n = 10, P < 0.001). DCG-IV, which at 300 nM has been demonstrated to be a selective group II agonist (Hayashi et al. 2017).
1993; Ishida et al. 1993), inhibited the field potentials by 23.3 ± 2.54% (n = 6, P < 0.001). In contrast, the specific group III agonist L-AP4 did not inhibit synaptic transmission, but at 25 μM actually produced a slight increase (6.07 ± 2.68%, n = 4, ns) in field potential amplitude. As with adenosine, the inhibition produced by DCG-IV was presynaptic, as indicated by the increased paired-pulse facilitation (data not shown).

In whole cell patch recordings of retinal ganglion cells under normal solution conditions, DCG-IV (500 μM) did not produce any decrease in cell input resistance (+2.54 ± 0.82%, n = 4) or any consistent change in membrane potential that could account for the presynaptic inhibition. Under voltage-clamp conditions that isolated calcium currents, however, DCG-IV potently inhibited calcium currents, consistent with its presynaptic inhibition of retinotectal synaptic transmission. 1S,3R-ACPD produced a smaller inhibition of calcium current, whereas L-AP4 had no effect. Sample recordings are shown in Fig. 3B, and the summary data are shown in Fig. 4A. DCG-IV at 300 nM inhibited the calcium current by 20.4 ± 3.0% (n = 6, P < 0.01). At 50 μM, 1S,3R-ACPD inhibited the calcium current by 12.3 ± 5.1% (n = 4, P < 0.05). In contrast, L-AP4 at 25 μM did not inhibit the calcium current (103 ± 1.6% of control; n = 3, P > 0.05). The inhibitory effect of DCG-IV was concentration dependent, as shown in Fig. 4B, with an IC₅₀ of <100 nM. The results indicate a selective inhibition by group II mGluRs of both synaptic transmission and calcium currents.

Adenosine and DCG-IV effects are mainly on N-type calcium channels

There are several types of calcium channels in goldfish retinal ganglion cells, as differentiated by the use of selective toxins (Bindokas and Ishida 1996). In our previous paper, we demonstrated that the synaptic transmission at goldfish retinotectal synapses is predominantly mediated by N-type calcium channels (100% inhibition by ω-Ctx GVIA, 0% by ω-AgaTx by 10.220.33.4 on June 17, 2017 http://jn.physiology.org/ Downloaded from

Voltage dependence of adenosine and DCG-IV inhibitions

We next explored whether the inhibition produced by adenosine and DCG-IV was due to a reduced amplitude of peak I_{Ca} or due to a shift of the current-voltage (I-V) curve to more depolarized membrane potentials, both of which could contribute to presynaptic inhibition by reducing calcium influx during the action potential. As shown in Fig. 5, it seemed that both mechanisms were involved in the inhibitory effects of adenosine and DCG-IV. In the presence of adenosine (100 μM) or DCG-IV (300 nM), the I-V curve of the calcium current was shifted upward and rightward, indicating both a decreased maximum current and a need for a greater depolarizing voltage to open the channels.
IVA). Here ω-Ctx GVIA at 0.5–1.0 μM reduced the peak calcium current from 300.5 ± 40 pA to 56.6 ± 13 pA, a decrease of 82.9 ± 2.03% (n = 8, P < 0.01, Fig. 6A), comparable with that observed by Bindokas and Ishida (1996). Adenosine (100 μM) then produced only a much smaller percentage inhibition of the remaining calcium current (5.3 ± 1.2%, n = 4, see Fig. 6B for sample records and Fig. 6D for summarized data). Similarly, DCG-IV after ω-Ctx GVIA produced only a small 4.9 ± 0.8% inhibition (n = 3, see Fig. 6C for sample records and Fig. 6D for summarized data). These findings demonstrate that the inhibitory effects of both adenosine and DCG-IV are mainly on the N-type calcium channels.

**Common mediation by PTX-sensitive G proteins**

Adenosine receptors and mGluRs belong to the superfamily of G protein–coupled receptors. Our previous paper has shown that adenosine-induced presynaptic inhibition of retinotectal synaptic transmission was sensitive to PTX. We thus tested whether the inhibitory effects of adenosine and DCG-IV on ganglion cell calcium current could be blocked by PTX. As shown in Fig. 7A, preincubation of ganglion cells with PTX (1 μg/ml, 4 h) did not in itself cause any noticeable decrease in the amplitude of the calcium currents (293 ± 26 pA vs. 303 ± 17 pA in controls), but it consistently resulted in a virtual elimination of the inhibition produced both by adenosine and by DCG-IV. The decrease in calcium current produced by 100 μM adenosine and by 300 nM DCG-IV in PTX-treated cells was 1.5 ± 0.3% (n = 5) and 3.3 ± 0.7% (n = 5), respectively, significantly smaller than those produced in normal, untreated cells (Fig. 7B). These results indicate that the inhibitory effects of both adenosine and DCG-IV on calcium currents were mediated by PTX-sensitive inhibitory G proteins.

**Common uncoupling by protein kinase C (PKC)**

PKC modulates cellular functions by phosphorylating a large array of cellular proteins, including membrane receptors, G proteins, and ion channels (Nishizuka 1986). Because we previously found that PKC activation uncoupled adenosine from presynaptic inhibition of field potentials, we tested whether PKC activation by TPA (a phorbol ester) or by (−)7-octylindolactam V (lactam), a nonphorbol PKC activator, also uncoupled both adenosine and DCG-IV from the inhibition of calcium currents in ganglion cells. Pretreatment of ganglion cells with TPA (1 μM) or lactam (300 nM) for 30 min...
greatly decreased the inhibitory effects of adenosine and DCG-IV (see Fig. 8 for sample records). The inhibition produced by 100 μM adenosine was 1.4 ± 0.28% (n = 5, P > 0.05) and 3.4 ± 0.8% (n = 7, P > 0.05) in the presence of TPA (1 μM) and lactam (300 nM), respectively (vs. 15.9 ± 2.6% inhibition in untreated cells). The corresponding values for DCG-IV inhibition were 5.1 ± 1.3% (n = 3, P > 0.05) and 3.3 ± 0.3% (n = 5, P > 0.05), respectively. TPA and lactam alone each had no significant effect on the membrane calcium current. Thus activation of PKC greatly decreased the inhibitory actions of both adenosine and DCG-IV.

Occlusion between adenosine receptor and mGLuR effects

Because the inhibitory effects on the calcium current produced by adenosine and DCG-IV were both similarly modulated by PKC and sensitive to prior PTX treatment, it seems likely that they share a common mechanism of action. On the other hand, PTX knocks out both Go and Gi, so they might work through different G proteins. To test this possibility, we performed occlusion experiments in which the application of adenosine was followed by DCG-IV, or in the reverse sequence (Fig. 9). The results showed that the inhibitory effects of adenosine and DCG-IV were not additive. No matter which agonist was applied first, the application of the second (with continued presence of the 1st) always produced a far smaller percent inhibition than that produced by the agonist when applied alone. Thus the inhibitions produced by adenosine and DCG-IV occluded each other, indicating that they share a common mechanism of action and act on the same population of N-type calcium channels.

DISCUSSION

Main findings of the present study

The present study compared the inhibitory effects of adenosine A1 receptors and mGluRs both on retinotectal synaptic transmission and on calcium currents in retinal ganglion cells. The results showed (1) that activation of adenosine A1 receptors and the group II mGluRs both inhibit retinotectal synaptic transmission in goldfish, (2) that this inhibition can be attributed to the inhibition of calcium current through the N-type calcium channels that were previously shown to mediate transmission, and (3) that the two inhibitory pathways share a common mechanism of action because they are both blocked by PTX inactivation of G-proteins, they are both uncoupled by C-kinase activation, and their effects occlude each other.

The results, taken together, suggest that the inhibition of calcium currents accounts for the mechanism of presynaptic inhibition by both adenosine and mGluRs. This is based on several lines of evidence. The first is the fact that neither adenosine nor DCG-IV decreased input resistance or hyperpolarized the retinal ganglion cells, thereby ruling out a strong activation of K channels as an alternative method of producing presynaptic inhibition. Second, there were many parallels between the effects on presynaptic inhibition and on calcium channels, including the same subclass of receptors (A1 adenosine and group II type of mGluRs), the same concentration...
dependence for agonists such as adenosine, CHA, and DCG-IV, and the same sensitivities to both PTX and PKC. Finally, the decreases in calcium current are sufficiently large, given the third or fourth power relationship between calcium concentration and transmitter release, to produce much larger percentage decreases in transmission. In a closer parallel, this exaggerated, nonlinear effect on transmission was also seen when N- or P-type subpopulations of calcium channels were blocked with selective toxins in mammalian CNS (Wheeler et al. 1994). Thus the calcium channel modulation is both large enough, and the only currently viable mechanism that can account for the presynaptic inhibition produced by adenosine A1 and group II mGluRs in this projection. Because these are the first findings in the visual system, this study extends the autoregulatory modulation of transmission to the goldfish retinotectal synapses where it may play a role both in shaping visual processing and in altering visual plasticity. We return to this latter point after discussing the components of the mechanism below.

**Heterogeneity of calcium channels**

By the use of ω-conotoxins and ω-agatoxins, at least four types of calcium channels, namely N, L, P, Q, have been identified as contributing to transmitter release in the CNS (Dunlap et al. 1995; Reuter 1996; Wheeler et al. 1994), although their relative contributions differ at different synapses. The goldfish retinotectal projection seems to be unique in CNS in its apparently exclusive reliance on N-type calcium channels. Although T-, L-, and N-type calcium channels are present in the goldfish retinal ganglion cells (Bindokas and Ishida 1996), we previously found that the N-type channel blocker ω-Ctx GVIA completely blocked retinotectal synaptic transmission, whereas the P-type blocker ω-agatoxin IVA had no effect, and the L-type blocker nifedipine slightly augmented transmission (Zhang and Schmidt 1998). Our present study provides evidence that the N-type calcium channels are the main targets of the inhibitory effects of both adenosine A1 receptors and the group II mGluRs, because after the N-type channels were blocked with ω-Ctx GVIA, both receptors produced much smaller percentage inhibitions of the small, remaining calcium current. The small effect most likely represents residual unblocked N-type current, as cost precluded using ω-Ctx GVIA above 0.5 μM in all but one case. In this regard, the goldfish retinal ganglion cells may prove to be a useful model for the study of calcium channel modulation by various neurotransmitters as well as pharmacological agents.

**Inhibition mediated by inhibitory G proteins**

Because the number of G protein subtypes is far less than the number of neurotransmitters, many neurotransmitters may utilize the same G proteins in their signal transduction pathways. The PTX-sensitive Gs and Gi proteins mediate most of the inhibitory effects on calcium channels, as they clearly do here. In neuroblastoma cell lines, the PTX-sensitive Gs and Gi proteins both converge on the N-type calcium channels, but Gs is capable of mediating only certain neurotransmitter effects and not others, which must therefore be ascribed to Gi (Taussig et al. 1992).

The present study showed that adenosine A1 receptors and the group II mGluRs may share the same PTX-sensitive G protein in producing inhibition of N-type calcium channels. This is suggested by the high degree of occlusion of the two inhibitory effects, and also by the ability of PKC to uncouple the inhibition. Only Gi is known to be inactivated by PKC phosphorylation (Katada et al. 1985), and group II mGluRs are known to inhibit adenylate cyclase by acting through Gs (Nakanishi 1994; Pin and Duvoisin 1995). Finally in sympathetic ganglia, PTX-sensitive inhibition, but not the separate PTX-insensitive inhibition of synaptic transmission, was modulated by PKC activation (Zhang et al. 1996), indicating an action of PKC at the G-protein.

**PKC modulation of the inhibition by adenosine receptors and mGluRs**

In the previous study, we observed that the inhibitory action of adenosine A1 receptors on retinotectal synaptic transmission could be blocked by activation of PKC with phorbol esters. The present study extended that finding by showing a similar block of the inhibition of calcium current following PKC activation with either the phorbol ester TPA or the nonphorbol activator lactam. The latter shows that this is not a result of non-PKC action of phorbols. In addition, the inhibitory action of the group II mGluRs on N-type calcium channels are similarly modulated by PKC activation. This PKC modulation is common to the inhibitory actions of many transmitters, especially those mediated by PTX-sensitive G-proteins (Shapiro et al. 1994; Swartz 1993; Zha and Ikeda 1994) and opens the process to control by other second-messenger pathways, providing a more flexible control of synaptic transmission. In the previous paper (Zhang and Schmidt 1998), we showed that PKC modulation of this inhibition may play a significant role in stabilizing the regenerating projection; responses at the immature synapses habituate extremely rapidly because of this inhibition, and PKC activation completely removes this habituation.

**Heterogeneity of mGluRs and inhibition of synaptic transmission**

In contrast to group I mGluRs, which are coupled to phospholipase C, group II and III mGluRs share similar signal transduction mechanisms, including the inhibition of adenylate cyclase (Pin and Duvoisin 1995). Previous studies have shown that activation of both group II and III mGluRs produce presynaptic inhibition of synaptic transmission, whereas group I mGluRs may augment transmitter release (Sanchez-Prieto et al. 1996; our unpublished results). Takahashi et al. (1996) reported that l-AP4, a group III agonist, inhibited presynaptic calcium current in the giant synapse (calyx of Held) of the rat (P-type calcium channels). However, in our present study, the group III mGluRs either inhibited transmission in the goldfish retinotectal synapse, nor inhibited calcium current in cultured retinal ganglion cells. The lack of group III mGluR effect in the present study presumably was due to the absence of group III receptors in ganglion cells because group II and III mGluRs work through the same G-protein (Gs). Although no study has investigated the expression of mGluRs in goldfish retinal ganglion cells, studies in other central neurons indicated that only a small percentage of neurons express all three groups of mGluRs (for example, see Chen and van den Pol 1998).
**Functional implications of presynaptic inhibition**

Findings from the present study extend our understanding on the physiology of retinotectal synaptic transmission and have implications for shaping of receptive fields, prevention of excitatory neurotoxicity, and control of plasticity. Excessive release of excitatory amino acids may result in neural damage, so it is of obvious importance that their release is controlled. Presynaptic inhibition of release by the same transmitter (autoregulation) or by other transmitters (heterologous regulation) is therefore an important mechanism for neural protection. One important finding made in our previous study (Zhang and Schmidt 1998) was that endogenous adenosine levels even in excised tectum are sufficient to inhibit transmission at the retinotectal synapse.

The present study demonstrated that the adenosine A1 receptor-mediated as well as the group II mGluR-mediated inhibition was due to depressing the N-type calcium channels, which have previously been shown to mediate synaptic transmission in the goldfish retinotectal synapses (Zhang and Schmidt 1998). Inhibition of calcium channels can be used either to depress excitatory inputs or to increase excitability by depressing inhibitory inputs (Stefani et al. 1994). Because we looked only at the excitatory retinal input, we do not know whether there is a similar control of inhibition in tectum. Such mGluRs on inhibitory retinal terminals can effectively alter processing by suppressing recurrent inhibition selectively at the active cells, thereby enhancing their signal relative to surrounding areas where lateral inhibition is unabated (Nakanishi 1994). Conversely, we earlier showed that there is a recurrent cholinergic circuit for presynaptic augmentation of retinal inputs to tectum (King and Schmidt 1991). Thus there are strong mechanisms to ensure the dominance of the strongest visual input at any one time. Autoregulation of excitatory inputs by mGluRs and adenosine A1 receptors can also help to explain the property of habituation to repeated visual presentations that is found in tectal but not lateral geniculate neurons (Niida et al. 1980; Oyster and Takahashi 1975).

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**REFERENCES**


