Effects of Metabotropic Glutamate Receptor Activation in Auditory Thalamus

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INTRODUCTION

Neurons in the ventral part of the medial geniculate body (MGBv) transform auditory input signals from the inferior colliculus en route to the auditory cortex. These neurons also receive a strong tonotopically organized input from the primary auditory cortex (Andersen et al. 1980; Morel and Imig 1987; Winer and Larue 1987; see Jones 1985 for a review). Both afferent sensory and corticothalamic inputs are glutamatergic in the medial and dorsal lateral geniculate bodies (Deschênes and Hu 1990; Hu et al. 1994; McCormick and von Krosigk 1992). However, only corticothalamic inputs activate metabotropic glutamate receptors (mGluRs), resulting in long-lasting excitatory responses (Eaton and Salt 1996; He 1997; McCormick and von Krosigk 1992). In various thalamic nuclei, postsynaptic type 1 mGluRs mediate these effects (Eaton and Salt 1996; Godwin et al. 1996a,b; Martin et al. 1992; Salt and Eaton 1996).

The activation of mGluRs can couple to a variety of effector mechanisms (see review by Pin and Duvoisin 1995). Synaptic stimulation or application of 1S,3R-1-aminocyclopentan-1,3-dicarboxylic acid (1S,3R-ACPD) can evoke a postsynaptic depolarizing current associated with decreased membrane conductance to K+ (e.g., Charpak and Gähwiler 1991; Charpak et al. 1990; Guérineau et al. 1994; McCormick and von Krosigk 1992). Other observed mechanisms include the activation of a Na+/Ca2+ exchanger (Keene et al. 1997; Linden et al. 1994; Staub et al. 1992) and Ca2+-dependent or -independent cation currents (Crépel et al. 1994; Guérineau et al. 1995; Mercuri et al. 1993; Zheng et al. 1995). In some neurons, the responses subsequent to activation of mGluRs have multiple components (Crépel et al. 1994; Guérineau et al. 1995; Keesee et al. 1997). In addition to these postsynaptic mechanisms, mGluRs have important roles in presynaptic modulation of transmission, synaptic plasticity, and neuronal death (see reviews by Nicoletti et al. 1996; Pin and Duvoisin 1995).

Despite serious implications for signal transduction in the auditory pathway, the effects of mGluR activation have not received examination in MGBv neurons. This report is the first description of mGluR-activated currents, frequency preferences, and 1S,3R-ACPD effects on firing patterns in MGBv neurons. Preliminary results have appeared in abstract form (Tennigkeit et al. 1996).

METHODS

The preparation of thalamic slices and recording conditions were similar to those described previously (Tennigkeit et al. 1996). The experiments followed a protocol approved by the Committee on
Animal Care of the University of British Columbia. Sprague-Dawley rats (16–21 days old) were decapitated during deep anesthesia with halothane. The brain was removed rapidly from the cranium and submerged in cold (−4°C) artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM) 124 NaCl, 26 NaHCO3, 10 glucose, 4 KCl, 2 CaCl2, 2 MgCl2, and 1.25 KH2PO4. The ACSF was saturated continuously with 95% O2-5% CO2, maintaining a pH of 7.3. Using a Vibraslicer (Campden Instruments, London, UK), we obtained coronal, 300-μm-thick slices that contained the medial geniculate body. After 3 h incubation of the slices at room temperature (22–25°C), we started the recording session.

Whole cell patch-clamp electrodes were pulled (Narishige, Model PP83) from borosilicate glass (WP-Instruments, Sarasota, FL). The electrode solution (pH 7.3) contained (in mM) 140 K-glucuronate, 10 HEPES, 5 KCl, 4 NaCl, 3 Na2ATP, 0.3 Na3GTP, 10 ethylene glycol-bis-(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), and 1 CaCl2 (~10 mM free Ca2+, calculated using Max Chelator software). In experiments with GTP analogues, 0.3 mM guanosine 5′-O-(3-thiotriphosphate) (GTPγS) or 0.3 mM 5′-O-(2-thiodiophosphate) (GDPβS) replaced GTP in the electrode solution. For solutions of low extracellular Na+ concentration (26 mM), we replaced NaCl with equimolar N-methyl-d-glucoside-Cl (NMDG). In the case of Li+ applications, we replaced 50 mM NaCl with equimolar LiCl. HEPES, bis-(o-aminophenoxy)-ethane-N,N,N′,N′-tetraacetic acid (BAPTA), EGTA, NMDG, 5-glutamate, tetrodotoxin (TTX), ATP (Na2ATP), GTP (GTP), GDPβS, GTPγS, and inorganic salts were purchased from Sigma (St. Louis, MO). In selected experiments, BAPTA (10 mM) replaced EGTA in the electrode solution.

Whole cell patch-clamp recordings were made with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) in current clamp or discontinuous single-electrode voltage-clamp mode (current-voltage switching frequency 4–5 kHz, 30% duty cycle, gain 3–5 nA/mV). Data acquisition, storage, and analysis were controlled using pClamp 5 software (Axon Instruments) and stored on videotape (super Beta, Sony). The experiments were recorded continuously on a chart recorder (Gould), digitized (PCM 501ES, Sony) and stored on videotape (super Beta, Sony). The voltage-current (V-I) relationships were determined with slow voltage-ramp protocols; neurons were continuously with 95% O2-5% CO2, maintaining a pH of 7.3. Using a Vibroslicer (Campden Instruments, London, UK), we obtained coronal, 300-μm-thick slices that contained the medial geniculate body. After 3 h incubation of the slices at room temperature (22–25°C), we started the recording session.

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The frequency responses of MGBv neurons were studied by injecting swept-sine wave (ZAP function) current inputs and Fourier transformation of the recorded voltage and current to the frequency domain, as described previously (Puil et al. 1986, 1994; Tennigkeit et al. 1997). The impedance (Z) amplitude profile (ZAP) is the ratio of this complex-valued impedance as a function of frequency in a range of 0.1–20 Hz, obtained from single ZAP records and smoothed with a five-point moving average (cf. Tennigkeit et al. 1997).

The ACSF was perfused at a flow rate of 2 ml/min (bath volume 0.3 ml). Ion channel blockers and antagonists were applied in the ACSF for a minimum of 10 min to allow a steady-state assessment of their effects. The following glutamate receptor agonists and antagonists were purchased from Precision Biochemicals (Virginia, BC): 1S,3R-ACPD, (RS)-α-methyl-4-carboxyphenylglycine (MCPG), α-2-amino-5-phosphonovalerate (d-APV), and 6-cyano-7-nitroquinoxline-2,3-dione (CNQX). In voltage-clamp experiments, TTX (300 nM) and Cd2+ (0.1 mM) were applied routinely to block action potentials and synaptic activity. In 10 experiments, 50 μM d-APV and 10 μM CNQX were applied throughout the experiment to block inotropic glutamate receptors.

Quantitative data are presented as means ± SE. Differences were evaluated using Student’s paired t-test and considered significant for P < 0.05.

RESULTS

Effects of 1S,3R-ACPD in MGBv neurons

The application of the selective mGluR agonist, 1S,3R-ACPD, depolarized all MGBv neurons (Fig. 1; n = 67). At the concentrations used (5–100 μM), the peak depolarization was in the range of 2–12 mV. On application of 50 μM to 15 neurons at ~60 mV, the depolarization reached threshold in 12 neurons, eliciting tonic firing of action potentials (Figs. 1A and 5). In 2 of the 12 neurons, the applications led to action potential bursting with plateau potentials (not shown) (cf. Klink and Alonso 1997; Tennigkeit et al 1997b; Zheng et al. 1995). Co-application of TTX (300 μM) with 1S,3R-ACPD completely abolished the action potentials and plateau potentials while only slightly reducing the peak amplitude of the evoked depolarization. The 1S,3R-ACPD response was well maintained despite repeated applications (Fig. 1B). Applications of S-glutamate (50 μM to 1 mM), in the presence of the ionotropic glutamate-receptor blockers, CNQX (10 μM) and APV (50 μM), also elicited depolarizations in two neurons (not shown). Recovery from the depolarization elicited by an application (30–60 s) occasionally was rapid but usually required 5–10 min (Fig. 1, A and B).

The amplitude of the 1S,3R-ACPD depolarization increased in a concentration-dependent manner. We investigated this dependence in 35 neurons in the presence of TTX (300 nM) to block voltage-dependent Na+-currents and Cd2+ (100 μM) to block high-threshold Ca2+-currents (cf. Tennigkeit et al. 1996). During their coapplication, a brief application of 1S,3R-ACPD (30 s in Fig. 1B) produced a depolarization that slowly increased in amplitude and decayed even more slowly. During an extended 1S,3R-ACPD application (7.5 min in Fig. 1B), we observed a small decline in the response (10–20%) over tens of seconds, possibly due to receptor desensitization. Full recovery occurred within 5–20 min (Fig. 1B) despite such long applications (4–8 min; n = 6). Brief 1S,3R-ACPD applications (50 μM for 30–60 s) were sufficient to elicit maximal depolarizing responses. We measured these peak amplitudes for estimates of the concentration-dependence, usually using only one concentration per neuron (n = 32). The concentration-response relationship in Fig. 1C appears to saturate close to 50 μM. The half-amplitude for a maximal effect occurred on applications of concentrations between 5 and 10 μM, close to the EC50 value for mGluR2 (Pin and Duvoisin 1995; Tanabe et al. 1992). For subsequent investigations of the mechanism of the depolarization, we chose 50 μM as the usual concentration for application.

Depending on the resting potential (Vr), the application of 1S,3R-ACPD induced a depolarization as well as a transformation of the firing pattern in response to injected current pulses. In neurons at a negative Vr (e.g., ~75 mV in Fig. 2A), a small depolarizing current pulse elicited low-threshold Ca2+ spike (LTS) burst firing before an 1S,3R-ACPD application.
During an 1S,3R-ACPD application, the depolarization increased in amplitude and the pulse-evoked firing pattern changed at first to firing of a single action potential with a delayed onset and then to tonic repetitive firing with a fast onset (Fig. 2A; n = 17). The same changes in the response pattern occurred during manual depolarization with DC when the neuron did not reach threshold during an application of 1S,3R-ACPD or in its absence (cf. Tennigkeit et al. 1996). However, when DC was used to hyperpolarize the neuron back to its original $V_R$, the response was reduced to a single action potential on top of a LTS, possibly due to a maintained depolarization and T-current inactivation in distal dendrites. We also observed similar reductions in the burst responses that occurred at the offset of hyperpolarizing current pulses in 10 of 17 neurons. We observed full recovery of these bursts in 7 of the 10 neurons within 15 min after terminating the 1S,3R-ACPD application.

**Effects of 1S,3R-ACPD on input conductance and impedance**

The applications of 1S,3R-ACPD did not significantly change input conductance as measured from the voltage responses to constant hyperpolarizing current pulses. The failure of 1S,3R-ACPD to change input conductance was confirmed on applying DC compensation to return the membrane potential back to the original $V_R$ (Fig. 1A). 1S,3R-ACPD also did not significantly change the slope of the $I-V$ relationship near rest. A small increase in input conductance ($5.4 \pm 2.8\%$; n = 12) occurred occasionally when the 1S,3R-ACPD depolarization evoked firing of action potentials, but we also observed similar increases without 1S,3R-ACPD application. When TTX application prevented the firing of action potentials, this increase in conductance was reduced to insignificant levels ($0.6 \pm 3.5\%$, n = 7; cf. Fig. 7A).

Because such conductance estimates rather poorly predict dynamic signal generation, we measured the impedance amplitude profiles (ZAP) (Puil et al. 1986) of MGBv neurons. At a $V_R$ of $-70$ mV, the neuron of Fig. 2 exhibited a voltage-dependent resonance near 1 Hz due to interaction of active currents, including the T-type Ca$^{2+}$ current, with the passive properties (Tennigkeit et al. 1994, 1997). After DC depolarization, the apparent impedance collapsed, resulting in low-pass filter characteristics (Fig. 2B, control). This occurred presumably as a result of excessive T-current inactivation, which prevented voltage oscillations at the resonance frequency (cf. Puil et al. 1994). The application of 1S,3R-ACPD dramatically reduced the impedances of MGBv neurons in a functionally important frequency range near 1 Hz, but this was mostly attributable to the depolarization. The frequency-response curves of Fig. 2B (middle) show that a DC hyperpolar-
Depolarization back to the initial $V_R$ reversed the shift from nonresonant to resonant behavior of the neuron. Hence the depolarization due to activation of mGluRs likely shifted the frequency responses of MGBv neurons from band-pass to low-pass filter properties.

**MGluR-antagonism**

The mGluR antagonist, MCPG (selective for the receptor subtypes, mGluR$_1$ and mGluR$_2$), reversibly blocked the depolarization induced by 1S,3R-ACPD. In the neuron of Fig. 3, for example, application of MCPG (0.5 mM of the racemic form) reduced the depolarization amplitude to 36.8% of the control. In a different neuron and in the presence of TTX and Cd$^{2+}$, the same concentration reduced the 1S,3R-ACPD depolarization to 44.4% of the control value. Application of MCPG alone did not evoke changes in membrane potential or input resistance. Using voltage clamp, we observed that 1S,3R-ACPD application evoked an inward current ($I_{ACPD}$) which an additional application (0.5 mM) of MCPG reduced to 43.8% of control (Fig. 3B). A similar blockade of 1S,3R-ACPD-evoked responses has

**FIG. 2.** 1S,3R-ACPD-evoked shifts in firing mode and frequency response characteristics. A: MGBv neuron ($V_R = -75$ mV) was depolarized by $-20$ mV on application of 1S,3R-ACPD (50 $\mu$M). Voltage responses to depolarizing current pulses (100 pA, 100 ms) changed from a low-threshold spike (LTS) burst (1) to delayed tonic (2) to fast-onset tonic firing (3) during the 1S,3R-ACPD depolarization. Hyperpolarization by injection of DC ($-90$ pA) to rest reveals only single-spike response on the LTS (4). B: in an MGBv neuron at rest ($-70$ mV), the impedance amplitude profile (ZAP) exhibits a resonant hump at $-1$ Hz. At a depolarized membrane potential ($-60$ mV, +DC injection), the frequency response shows low-pass filter characteristics. During application of 1S,3R-ACPD (50 $\mu$M), the membrane depolarized to $-60$ mV and shifted the frequency response to a low-pass function. Negative DC injection to $-70$ mV reversed this shift. After termination of the application, membrane potential and frequency responses recovered to control values.

**FIG. 3.** (RS)-$\alpha$-methyl-4-carboxyphenylglycine (MCPG) blocked 1S,3R-ACPD effects in MGBv neurons. A: application of MCPG (0.5 mM) reversibly blocked the depolarization induced by 1S,3R-ACPD (50 $\mu$M). Break between control and MCPG represented 10 min, and 35 min passed before the recovery record. B: MCPG blocked $I_{ACPD}$, recorded in voltage-clamp in the presence of TTX and Cd$^{2+}$. Break between the records represents 10 min.
been observed in other thalamic neurons (cf. Salt and Eaton 1996). The partial blockade in MGBv neurons is consistent with the known potencies of MCPG at mGluR1a (EC$_{50}$ 5 mM) and mGluR2 (EC$_{50}$ 500 mM) receptors.

Mediation by G proteins

We considered the possibility that G proteins mediated the 1S,3R-ACPD response (Pin and Duvoisin 1995). On replacement of GTP in the recording electrode with the nonhydrolysable GTP analogues, GTP$_{yS}$ or GDP$_{bS}$ (either at 0.3 mM), we proceeded to record $I_{ACPD}$. When GTP$_{yS}$ was being applied internally, the application of 1S,3R-ACPD irreversibly activated $I_{ACPD}$ (Fig. 4A; $n$ = 3). During such observations of $I_{ACPD}$ (recorded for ≤20 min), a second 1S,3R-ACPD application had no effect (Fig. 4A). The magnitude of $I_{ACPD}$ was 148.3 ± 23.9 pA or 76.2 ± 28.4% (n = 3) greater than the averaged $I_{ACPD}$ amplitude without GTP$_{yS}$ (84.2 ± 5.4 pA, n = 22; cf. Fig. 9D). In contrast, internally applied GDP$_{bS}$ reduced $I_{ACPD}$. This reduction was more pronounced during longer periods of recording and hence, longer applications of GDP$_{bS}$ ($n$ = 3). The maximal amplitude recorded 10 min after breaking through the cell membrane, for example, was 65.7 ± 3 pA, i.e., 22.0 ± 3.5% less than the average control $I_{ACPD}$ (Fig. 4B). When measured 20–30 min after breakthrough, the maximal amplitude of $I_{ACPD}$ was 32 ± 1.7 pA, corresponding to a reduction of 62.0 ± 2.1% (n = 3). The above results implicate an involvement of G proteins in the activation of $I_{ACPD}$ and a possible role for GTP hydrolysis in its deactivation.

Na$^+$ dependence of $I_{ACPD}$ and depolarization

Although 1S,3R-ACPD application did not reduce input conductance, we occasionally found that it slightly increased input conductance. Hence we examined the possibility that an activation of a highly voltage-dependent inward current, rather than blockade of outward K$^+$ currents, mediated the depolarization (cf. McCormick and von Krosigk 1992). For a test of
Na⁺ involvement, we reduced the extracellular Na⁺ concentration from 150 to 26 mM (“low Na⁺”) in two neurons. These conditions resulted in several reversible changes in both neurons: hyperpolarization (2 and 5 mV), reduced rate of rise and amplitude as well as increased duration of action potentials, decreased amplitude of the LTS, and reduced inward rectification at hyperpolarized potentials. After DC compensation of the hyperpolarization, the low Na⁺ conditions reversibly reduced the 1S,3R-ACPD-evoked depolarization to 10.7 and 14.1% of the control responses (Fig. 5). We attributed the low Na⁺ effects to reductions of the fast and persistent Na⁺- and hyperpolarization-activated cation (Iₚ) currents.

To investigate the possibility that a reduction in the persistent Na⁺ current could have contributed to the blockade of the 1S,3R-ACPD-evoked depolarization, we studied the effects of low Na⁺ conditions and TTX application on the ACPD depolarization. Alone the combined attenuation of Na⁺ currents evoked a hyperpolarization of 2.8 ± 1.1 mV (n = 5) and, in voltage clamp, a steady-state outward current of 48 ± 21.8 pA (n = 5). These conditions also reversibly reduced the control 1S,3R-ACPD-evoked depolarization by 70.4 ± 9.4% (n = 4, not shown) or, with the additional application of Cd²⁺, by 67.2 ± 10.3% (Fig. 6; n = 5, Vₘ = −70 mV). Using a voltage-ramp command (see METHODS), during combined TTX and Cd²⁺ application, we recorded an inward Iᵦ, with a V-I relationship that was nearly parallel to the voltage-axis between −40 and −85 mV (cf. Figs. 6–11, controls), reflecting an unchanged input conductance (n = 29). Throughout this voltage range, the lower external [Na⁺] greatly reduced Iᵦ (Fig. 6B). These results implicate a Na⁺-dependent current in the 1S,3R-ACPD-evoked depolarization.

Is a Na⁺/Ca²⁺ exchanger involved in Iᵦ?

The activation of electrogenic transport or exchange mechanisms could account for the Na⁺-dependent inward current produced by 1S,3R-ACPD application. In amygdala neurons, for example, mGluRs mediate an activation of a Na⁺/Ca²⁺ exchange current (Keele et al. 1997). Because Li⁺ ions cannot replace Na⁺ in this exchange mechanism but penetrate Na⁺ and nonselective cation channels, we studied the effects of partial replacement of Na⁺ in the ACSF on the Iᵦ (cf. Crépel et al. 1994). Potentially, equimolar replacement of Na⁺ with 50 mM Li⁺ could reduce a Na⁺/Ca²⁺ exchange current by ~65% (cf. Keele et al. 1997). In MGBv neurons, Li⁺ replacement by itself caused a reversible depolarization of 4.8 ± 1.2 mV (n = 4) and a steady-state inward current (Fig. 7B). This effect may be partly attributable to inhibition of Na⁺/K⁺-ATPase (Padjen and Smith 1983). Under Li⁺ replacement conditions, however, the Iᵦ in the neuron of Fig. 7, measured at −70 mV, was maintained at 93% of the control peak amplitude. In four neurons, the 1S,3R-ACPD-evoked depolarization remained unchanged in amplitude (8.3 ± 0.6 mV or 99.8 ± 12.1% of control). Li⁺ replacement or 1S,3R-ACPD application also reduced inward rectification below −90 mV in a reversible manner (Fig. 7, controls). The results imply that Li⁺ can replace Na⁺ as a charge carrier for Iᵦ and were not consistent with a major involvement of a Na⁺/Ca²⁺ exchanger in the 1S,3R-ACPD depolarization from rest (cf. Crépel et al. 1994; Keele et al. 1997) in MGBv neurons.

![Figure 6](http://jn.physiology.org/). Blockade of Iᵦ by low extracellular [Na⁺] conditions. A: under voltage-clamp conditions, 1S,3R-ACPD (50 μM) application evoked an inward current (inset, Vₘ = −65 mV). Current responses to hyperpolarizing voltage ramps before and after application of 1S,3R-ACPD revealed the Iᵦ by subtraction. V-I relationship of Iᵦ was almost parallel to the V axis between −85 and −50 mV with an outward slope less than −90 mV. B: low extracellular [Na⁺] conditions reduced Iᵦ. Note absence of slope at hyperpolarized potentials. C: near full recovery occurred after return to control artificial cerebrospinal fluid.
Are $I_{ACPD}$ and the depolarization influenced by $Ca^{2+}$?

Replacement of $Ca^{2+}$ with Mg$^{2+}$ in the ACSF did not significantly change the 1S,3R-ACPD depolarization. In the presence of TTX and Cd$^{2+}$, the depolarization was 94.3 ± 4.6% of control ($n=3$, Fig. 8A). Previous studies (Keele et al. 1997) have shown that replacement of $Ca^{2+}$ with 10 mM Mg$^{2+}$ together with application of EGTA (1 mM) in the ACSF blocked an 1S,3R-ACPD-evoked inward current that had a $V-I$ relationship that is similar to that described here. However, these conditions reversibly increased $I_{ACPD}$ by 18.7% ($V_m=-70$ mV, Fig. 8, B and C). Hence, voltage-dependent $Ca^{2+}$ currents did not seem to provide a major or specific contribution to $I_{ACPD}$.

The intracellular application of BAPTA (10 mM) by diffusion from the recording electrode caused a time-dependent reduction of the 1S,3R-ACPD depolarization and $I_{ACPD}$. On coapplication of TTX and Cd$^{2+}$ to two neurons, the mean 1S,3R-ACPD depolarization was reduced by 49.4 and 48.1% at 12 or 30 min of BAPTA application, respectively. Under voltage-clamp conditions and continuous 1S,3R-ACPD application, the $I_{ACPD}$ was present for 10 min before declining to an undetectable amplitude after 30 min. This decline in amplitude may have resulted from processes far from the electrode tip (see also preceding text). One possibility is that a rise in internal $[Ca^{2+}]_i$ in the dendrites may activate or potentiate the $I_{ACPD}$ and depolarization.

Does a $K^+$ current blockade contribute to $I_{ACPD}$?

A combined activation of inward and blockade of outward currents could have resulted in the parallel $V-I$ relationships (cf. Crépel et al. 1994; Shen and North 1992). As a test of this possibility for $I_{ACPD}$, we studied the effects of changes in extracellular $[K^+]_e$ and the $K^+$-channel blockers, Ba$^{2+}$ and Cs$^+$. A lowering of extracellular $[K^+]_e$ from the normal, 5.25, 724 F. TENNIGKEIT, D.W.F. SCHWARZ, AND E. PUIL
to 2.5 mM did not cause significant or specific alterations in $I_{\text{ACPD}}$ (Fig. 9, $A$–$D$; $n = 3$, $V_m = -70$ mV). Figure 9D shows the changes in $I_{\text{ACPD}}$ amplitude under control, $K^+$-channel blockade, and reduced extracellular $[K^+]_o$ and $[Na^+]_o$-conditions. In 9 of 20 neurons, $I_{\text{ACPD}}$ was nearly voltage-independent below $-40$ mV (Figs. 9A and 10A).

Application of $Ba^{2+}$ (0.1–1 mM) did not significantly change $I_{\text{ACPD}}$ (97.7 ± 4.2% of control; $n = 3$, $V_m = -70$ mV, Fig. 10B). The current induced by $Ba^{2+}$, however, outwardly rectified at negative potentials and was roughly linear down to its reversal near the calculated $E_K$ ($-85$ mV). This implied that $Ba^{2+}$ blocked an inward rectifier and a leak $K^+$ current. The blockade of inward rectifiers by $Cs^+$ (2.5–3 mM) or coapplication of $Ba^{2+}$ and $Cs^+$ (Fig. 10C) gave similar results—no significant effects on $I_{\text{ACPD}}$ (107.7 ± 8.4% of control; $n = 3$, $V_m = -70$ mV). In summary, the application of $Ba^{2+}$, $Cs^+$, and reduced extracellular $[K^+]_o$ produced only small changes in $I_{\text{ACPD}}$ and its V-I relationship. We concluded that a blockade of $K^+$ currents does not cause the generation of the $I_{\text{ACPD}}$ (between $-40$ and $-85$ mV).

**MGLuRs mediate a blockade of hyperpolarization-activated rectification**

The V-I relationship of the $1S,3R$-ACPD-induced current had a striking voltage dependence in the hyperpolarized range beyond $-85$ mV (Fig. 11A, bottom), leading to a reversal at $-101.2 ± 1.7$ mV within the investigated voltage range (−130 to −10 mV) in 6 of 11 neurons. Application of $1S,3R$-ACPD reduced the inward rectification, negative to $E_K$ (Fig. 11A, top). The application of extracellular $Ba^{2+}$ (0.1 mM, $n = 1$; not shown) or $Cs^+$ (2.5 mM; Fig. 11B, $n = 3$) completely blocked the rectification and occluded the voltage dependence. This implicated a blockade of an inwardly rectifying $K^+$ current, $I_{KIR}$, during mGluR activation. Partial replacement of $Na^+$ in the ACSF also reversibly eliminated the outward rectification in the hyperpolarized range (Fig. 6, $n = 6$). A mGluR-dependent blockade of a hyperpolarization-activated cation current ($I_H$) was difficult to ascertain because $I_{KIR}$ dominated the hyperpolarization activated inward rectification, masking $I_H$ in MGBv neurons (Tenniglet et al. 1996). However, the above results are consistent with a mGluR-mediated blockade of hyperpolarization-activated currents, including $I_{KIR}$.

**Coactivation of a voltage-gated outward current with the $I_{\text{ACPD}}$**

Using blockade of $Na^+$ and $Ca^{2+}$ spikes by TTX and $Cd^{2+}$ and large voltage ramps, we explored the effects of mGluR activation in the depolarized voltage range. At potentials positive to $-40$ mV, the whole cell current induced by $1S,3R$-
results is that, in the depolarized range, 1S,3R-ACPD activated voltage-dependent, outward K\(^+\) and inward cation currents, the latter with close similarities to the TTX resistant, persistent Na\(^+\) current (Cummins and Waxman 1997), in MGBv neurons.

**Discussion**

The application of 1S,3R-ACPD to MGBv neurons had three distinct effects. First, we observed an inward current (\(I_{\text{ACPD}}\)) that was sensitive to blockade with a specific antagonist of mGluR activation. The consequences of the depolarization were shifts in subthreshold filtering from resonance to low-pass characteristics and signal generation from burst to tonic patterns. The other effects were a blockade of an inwardly rectifying current in the hyperpolarized range and an activation of a K\(^+\) current at suprathreshold potentials.

**Ionic mechanism(s) of \(I_{\text{ACPD}}\)**

To all appearances, \(I_{\text{ACPD}}\) was independent of voltage throughout a wide range of membrane potentials (−40 to −85 mV). This led us to consider that, like other mGluR-activated cationic currents (cf. Keele et al. 1997; Mercuri et al. 1993), \(I_{\text{ACPD}}\) may participate in the regulation of \(V_R\) in MGBv neurons. However, it seemed unlikely that a blockade of voltage-independent K\(^+\) channels (Crépel et al. 1994; McCormick and von Krosigk 1992; Womble and Moises 1994) produced \(I_{\text{ACPD}}\) because 1S,3R-ACPD application did not reduce input conductance, and we could observe \(I_{\text{ACPD}}\) during pharmacological blockade of K\(^+\) channels.

The first clue that the ionic mechanism involved Na\(^+\) came from our observations of the effects of TTX blockade. Whereas \(I_{\text{ACPD}}\) survived the blockade, the 1S,3R-ACPD depolarization decreased in amplitude, as expected from a blockade of persistent Na\(^+\) current in MGBv neurons (Tennigkeit et al. 1997). Indeed, we found that \(I_{\text{ACPD}}\) depended on the extracellular [Na\(^+\)] despite the blockade of voltage-gated Na\(^+\) channels. This bears resemblance to the mGluR-activated current in amygdaloid neurons (Keele et al. 1997) and Purkinje cells (Staub et al. 1992), produced by Na\(^+\)/Ca\(^2+\) exchange. For the mGluR activation in MGBv neurons, however, such an exchange mechanism seemed less likely because we found that Li\(^+\) can replace Na\(^+\) as a charge carrier for \(I_{\text{ACPD}}\). Although we cannot entirely exclude an involvement of Na\(^+\)/Ca\(^2+\) exchange, the more likely possibilities are that 1S,3R-ACPD application caused a small increase in a voltage-dependent Na\(^+\) conductance (insensitive to blockade with 300 nM TTX) or acted on distal dendritic receptors to produce the depolarizing current.

We investigated a possible contribution of a mGluR-activated, Ca\(^2+\)-sensitive nonselective cation current, \(I_{\text{CAN}}\), as in hippocampal (Crépel et al. 1994) and locus coeruleus neurons (Shen and North 1992). There was some similarity because intracellular application of the Ca\(^2+\) chelator, BAPTA, also suppressed \(I_{\text{ACPD}}\) in MGBv neurons. However, a marked conductance increase, voltage dependence, and an insensitivity to MCGP blockade distinguished this current from the \(I_{\text{ACPD}}\) in MGBv neurons.

We observed that \(I_{\text{ACPD}}\) became dependent on voltage in the range positive to −40 mV. Here, a Cs\(^+\) blockade of a K\(^+\)
current, which coactivated with $I_{\text{ACPD}}$ unmasked a voltage dependence that resulted in a peak amplitude near $-30$ mV. This was reminiscent of the TTX-insensitive, persistent Na$^+$ current in C-type dorsal root ganglion neurons (Cummins and Waxman 1997).

In summary, $I_{\text{ACPD}}$ in MGBv neurons likely results from an increase in Na$^+$ conductance that may depend on internal Ca$^{2+}$ for activation. A persistent Na$^+$ current enhances this depolarizing action, which, itself, may be highly voltage dependent or mostly of dendritic origin. At voltages above threshold, a K$^+$ current can limit the extent of the 1S,3R-ACPD depolarization. This represents a novel sequence of mechanisms for mGluR-dependent excitation of thalamic neurons.

**Receptors mediating $I_{\text{ACPD}}$**

As suggested in the preceding text, the characteristics of $I_{\text{ACPD}}$ in MGBv neurons are consistent with a dendritic location of greatest mGluR density (Jones and Powell 1969; Vidnanszky et al. 1996). An abundance of mGluR1 protein occurs in rat MGBv (Fotuhi et al. 1993; Martin et al. 1992; Salt and Eaton 1996). In other thalamic nuclei, there are descriptions of the mGluR1 mediation of postsynaptic responses (Eaton and Salt 1996; Godwin et al. 1996a,b). The observed partial blockade of $I_{\text{ACPD}}$ with MCPG (0.5 mM) would exclude a mGluR5 mediation of $I_{\text{CAN}}$, as in CA1 pyramidal neurons (Congar et al. 1997) but is compatible with an involvement of mGluR1a (EC$_{50}$ = 300 µM) or mGluR2 (EC$_{50}$ = 500 µM). The depolarization of MGBv neurons caused by 1S,3R-ACPD application saturated at 50 µM. This concentration is a rather low for an involvement of mGluR1 (EC$_{50}$ = 105-170 µM) and entirely compatible with a role of mGluR2 (EC$_{50}$ = 5 µM). Thus while different receptors may mediate the 1S,3R-ACPD effects on hyperpolarization-activated rectification and the depolarization-activated K$^+$ current, the candidates for $I_{\text{ACPD}}$ mediation are type 2 or 1 mGluRs.

**Intracellular signal transduction**

Using the GTP analogues, GTPyS and GDPβS, we demonstrated an involvement of G proteins in mGluR-mediated signal transduction for the $I_{\text{ACPD}}$. While expecting G-protein activation for "metabotropic" receptors, there is a report that 1S,3R-ACPD activation of a cationic current may not involve G proteins but instead novel or membrane-delimited intracellular messenger systems (Guérineau et al. 1995). In MGBv neurons, intracellular application of GTPyS irreversibly activated $I_{\text{ACPD}}$, locking G proteins in an activated position (cf. Ross 1989). Similar application of GDPβS reduced $I_{\text{ACPD}}$ with a delay, attributable to diffusion into the distal dendrites, the well-known location of mGluRs and corticothalamic synapses (Godwin et al. 1996a; Jones and Powell 1969; Vidnanszky et al. 1996). Normally, the depolarization and $I_{\text{ACPD}}$ outlasted the brief 1S,3R-ACPD application by several minutes; this is consistent with G-protein activation of intracellular messenger systems (cf. Pin and Dulovsoin 1995).

An intracellular Ca$^{2+}$ signal appears to mediate the link between the receptor-activation and $I_{\text{ACPD}}$, although the source of the signal remains obscure. Internal application of BAPTA reduced $I_{\text{ACPD}}$ after a delay that could have resulted from slow diffusion of the chelator and/or slow Ca$^{2+}$-modulated processes. The signal does not apparently depend on Ca$^{2+}$ influx into the neuron because $I_{\text{ACPD}}$, activated during Ca$^{2+}$-channel blockade with Cd$^{2+}$, remained unchanged in low [Ca$^{2+}$] and high [Mg$^{2+}$] media. An intracellular Ca$^{2+}$-release from inositol trisphosphate (IP3)-sensitive stores is compatible with a role of mGluR1s, which are colocalized with IP3 receptors in rat MGB (Fotuhi et al. 1993). For example, a rise in intracellular [Ca$^{2+}$], mediated by mGluR1 activation in thalamus, has been implicated in seizure generation and neurotoxicity (McDonald et al. 1993; Tizzano et al. 1995; see review by Nicoletti et al. 1996).

**Modulation of the inward rectifier**

About 50% of MGBv neurons displayed a prominent inward rectification below $-85$ mV, which was reduced by application of 1S,3R-ACPD. A Cs$^+$ or Ba$^{2+}$ blockade of the rectifying current, $I_{\text{KIR}}$ (Tennigk et al. 1996; cf. Womble and Moises 1993) occluded this 1S,3R-ACPD action. A similar occlusion occurred after partial replacement of external Na$^+$ with Li$^+$, possibly relating to an interdependence of Na$^+$ and K$^+$ in the $I_{\text{KIR}}$ channel function (Hille 1992). A reduction of $I_{\text{KIR}}$ may result from a dilution of soluble intracellular mediators during whole cell recording. It is not surprising, therefore, that we did not observe the inward rectification and its modification by 1S,3R-ACPD application in all neurons.
A mGluR-dependent $K^+$ current at depolarized potentials

On application of 1S,3R-ACPD, an outward current that was sensitive to Cs$^+$ blockade was coactivated with the $I_{\text{ACPD}}$ at potentials positive to $-40$ mV. This implies an endowment of MGBv neurons with a mGluR-activated, voltage-dependent $K^+$ current, possibly activated by Ca$^{2+}$ (cf. Budde et al. 1992). A balance between the outward current and the inward $I_{\text{ACPD}}$ may have contributed to the apparent voltage independence (cf. preceding text) of the whole cell current activated by 1S,3R-ACPD.

Functional significance

The depolarization due to $I_{\text{ACPD}}$ dramatically changed the functional behavior of MGBv neurons. Their response mode shifted from resonant or oscillatory bursting to low-pass filter behavior and tonic firing as a direct result of the depolarization. This resembles mGluR-dependent behavior in the dorsal lateral geniculate nucleus (Godwin et al. 1996b; McCormick and von Krosigk 1992) and the effects of muscarinic agonists or raised extracellular [K+] in MGBv neurons (Hu 1995; Mooney et al. 1995). The transformation in firing pattern characterizes the transitions from sleep or drowsiness to states of alertness which correlate to the release of several neuromodulators, including acetylcholine and glutamate (McCormick 1992; Salt and Eaton 1996; Steriade and Llinás 1988). Modulators may interact at the cellular level, sharing G-protein-controlled messenger pathways (cf. Ross 1995) or converge with mGluR activation of the same effector systems, as in hippocampal neurons (Guérineau et al. 1994, 1995). Such mGluR and muscarinic interactions may regulate the leak $K^+$ current in the dorsal lateral geniculate nucleus (McCormick 1992). Alternatively, the depolarization due to modulatory mechanisms may invoke the tonic firing mode in different functional contexts. For example, muscarinic $K^+$-conductance blockade may promote a general state of alertness, whereas the corticofugal mGluR-dependent modulation may focus attention during recognition of a visual signal in background (Sherman and Koch 1986; Sillitto et al. 1994; Singer 1977). In the auditory thalamus, an excitatory input of long duration from the cortex enhances frequency tuning (He 1997; Villa et al. 1991; Zhang et al. 1997), presumably by mGluR activation.

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