Activation of a Nonselective Cationic Conductance by Metabotropic Glutamatergic and Muscarinic Agonists in CA3 Pyramidal Neurons of the Rat Hippocampus

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We have characterized a cationic membrane conductance activated by metabotropic glutamatergic and muscarinic cholinergic agonists in CA3 neurons in hippocampal slice cultures using the patch-clamp technique. When the potassium concentration in the superfusing fluid was raised above 5 mM, a biphasic current was observed in cells held at -60 mV in response to stimulation of postsynaptic metabotropic glutamate receptors (mGluRs) with 1S,3R-ACPD (50 μ M) or muscarinic receptors with methacholine (MCh, 5 μ M). The initial inward component was due to an increase in a cationic membrane conductance as determined by its reversal potential and its sensitivity to changes in extracellular K^+ or Na^+ . The conductance underlying this current displayed no apparent voltage sensitivity over the range -120 to -50 mV. The response was reduced by extracellular application of Ba^{2+} , Cd^{2+} , Mg^{2+} , or TEA, whereas extracellular Cs^+ or loading cells with BAPTA or Cs^+ did not affect the current. The effects of 1S,3R-ACPD were reversibly inhibited by bath-applied MCPG, an antagonist at mGluRs. Experiments with atropine and pirenzepine indicated that non- M_1 muscarinic receptors mediated the MCh-induced current. A decrease in a resting leak potassium conductance ($I_{K,leak}$) was responsible for the late component of the 1S,3R-ACPD- and MCh-induced response, seen as an outward current in the bathing solution with high K^+ concentration. Loading cells with GDP β S, GTP γ S, or GTP did not alter the cationic current, while, in the same cells, the reduction in $I_{K,leak}$ was abolished or irreversibly activated. Single-channel recordings of cationic channel activity in the cell-attached configuration provided evidence for the requirement of second messengers in coupling these receptors to the cationic channels.

The data indicate that in addition to the previously described reduction of $I_{K,leak}$, I_{M_1} , and I_{AHP} , both 1S,3R-ACPD and MCh activate a nonselective cationic conductance that is clearly revealed upon elevating external K^+ concentration. This current is mediated by activation of metabotropic

receptors, although no evidence could be obtained to show an involvement of G-proteins.

[Key words: metabotropic glutamate receptors, muscarinic cholinergic agonist, nonselective cationic conductance, elevated extracellular K^+ concentration, G-proteins, single-channel recordings, hippocampal slice cultures]

Metabotropic glutamate receptors (mGluRs) and muscarinic acetylcholine receptors play a crucial role in the control of excitability in the CNS by modulating the electrical behavior of neurons due to their coupling to numerous membrane ionic conductances (for reviews, see Nicoll et al., 1990; Gerber and Gähwiler, 1994). Electrophysiological studies have demonstrated that activation of postsynaptic mGluRs in hippocampal neurons results in a depolarization associated with an increase in membrane resistance, a reduction of action potential accommodation, and a blockade of the slow calcium-dependent afterhyperpolarization (Stratton et al., 1989, 1990; Baskys et al., 1990; Chrapak et al., 1990; Desai and Conn, 1991). Voltage-clamp experiments have revealed that the depolarizing response underlying the stimulation of mGluRs is essentially the consequence of a suppression of at least three K^+ currents: the voltage-dependent current I_M , the slow calcium-dependent afterhyperpolarizing current I_{AHP} , and a voltage-independent leak current $I_{K,leak}$ (Chrapak et al., 1990; Chrapak and Gähwiler, 1991; McCormick and von Krosigk, 1992; Guérineau et al., 1994). These K^+ currents are similarly diminished in the presence of muscarinic cholinergic agonists (Dodd et al., 1981; Halliwell and Adams, 1982; Lancaster and Adams, 1986; Madison et al., 1987). In addition, previous work has shown that cholinergic atropine-sensitive receptors mediate the activation of nonselective cationic conductances in the hippocampus (Segal, 1982; Benson et al., 1988; Colino and Halliwell, 1993), in neurons of the locus ceruleus (Egan and North, 1985), in neocortical interneurons (McCormick and Prince, 1985, 1986), and in sympathetic ganglion cells (Jones, 1985). To date, it is not clear whether mGluRs gate a similar cationic conductance. Such a conductance may be involved in generating a slow afterdepolarization in response to stimulation of mGluRs in hippocampal, septal, and cortical neurons (Constanti and Libri, 1992; Greene et al., 1992; Zheng and Gallagher, 1992; Caeser et al., 1993) and a depolarization in cerebellar Purkinje cells (Staub et al., 1993).

The purpose of this study was to determine whether activation of muscarinic receptors and mGluRs can induce a cationic current in CA3 pyramidal neurons in rat hippocampal slice cultures, and, if so, to characterize the second messengers involved.

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Materials and Methods

Organotypic hippocampal slice cultures were prepared as previously described (Gähwiler, 1981). Briefly, hippocampi were removed under aseptic conditions from 6-d-old Wistar rats that had been killed by decapitation. Slices (400 μ m thick) were embedded on glass coverslips in a film of clotted chicken plasma, transferred to test tubes containing semisynthetic medium, and then placed in a roller drum (10 revolutions per hr) in an incubator at 36°C. The culture medium was composed of 50% Eagle's basal medium, 25% balanced salt solution containing Hanks' or Earle's salts, and 25% heat-inactivated horse serum with glucose 33.3 mM. After 15–30 d *in vitro*, slices were transferred to a recording chamber attached to the stage of an inverted microscope fitted with differential interference contrast optics and continuously superfused with an extracellular solution at 25°C containing (in mM) 137 NaCl, 2.7 KCl, 2.8 CaCl₂, 2 MgCl₂, 11.6 NaHCO₃, 0.4 NaH₂PO₄, and 5.6 glucose, which was brought to pH = 7.3 by bubbling with CO₂. When the concentration of KCl was raised (up to 16 mM), NaCl content was correspondingly reduced. In some experiments, [Na⁺]_o was modified by substituting *N*-methyl-D-glucamine chloride for NaCl in equimolar amounts. Tetrodotoxin (TTX, 0.5 μ M) was added to the superfusing fluid to block propagated electrical activity and to reduce transmitter release.

Electrophysiological experiments were performed on CA3 pyramidal neurons using the patch-clamp technique in the whole-cell configuration or the cell-attached configuration (Hamill et al., 1981). Patch pipettes were pulled to a resistance of 2–5 M Ω from borosilicate glass (1.5 mm outer diameter, 1.17 mm inner diameter) and filled with the following internal solution for whole-cell patch recordings (in mM): 140 potassium gluconate, 10 KCl, 2 MgCl₂, 1.1 EGTA, 5 HEPES, that was titrated to pH = 7.2 with KOH. In some experiments, K⁺ ions were replaced by Cs⁺ ions (140 mM Cs⁺-gluconate in the patch pipette), and the pH was adjusted with CsOH. Some cells were loaded with the Ca²⁺ chelator BAPTA (20 mM) by passive diffusion from the patch pipette. Membrane currents were recorded under voltage-clamp conditions using a List EPC-7 amplifier (List Instruments, Darmstadt, Germany), filtered at 1 kHz and digitized at 1 kHz. Junction potentials were compensated after placing the pipette in the bath. Electrodes were advanced a few microns until they just deformed the membrane of visually identified CA3 neurons, while maintaining a high positive pressure on the back of the pipette. High resistance seals (> 1 G Ω) were obtained by applying negative pressure. The membrane beneath the pipette was then ruptured with strong negative pressure to achieve continuity with the intracellular compartment. Patch-clamp signals were fed into separate channels of an analog to digital converter (TL-1/DMA interface, Axon Instruments, Inc., Foster City, CA), digitized, stored, and analyzed on a PC using pCLAMP software. Voltage pulse generation, data acquisition, and analysis were performed with the same software/hardware system. For single-channel recordings, the patch pipette was filled with the same solution as that used externally, containing 16 mM K⁺ and 0.5 μ M TTX. Unitary currents were amplified with an Axopatch 200A (Digidata 1200 interface, Axon Instruments) and stored on a Panasonic digitizing recorder before off-line analysis using pCLAMP software (version 6.0.1, Axon). Data was sampled at a rate of 5 kHz and filtered with a cut-off frequency of 1.5 kHz.

Drugs were applied in the bathing fluid for whole-cell recordings and by fast close superfusion for cell-attached recordings. For our fast superfusion system, we have calculated a dilution factor of at least 2.5 and therefore higher agonist concentrations were used to fill the ejection pipette in the cell-attached experiments. Drugs were purchased from the following sources: 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD), the enantiomer 1R,3S-ACPD, (RS)- α -methyl-4-carboxyphenylglycine (MCPG), 6-cyano-7-nitroquinoline-2,3-dione (CNQX), quisqualate, L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), and D-2-amino-5-phosphonovalerate (D-APV) from Tocris Neuramin (Bristol, UK); TTX from Sankyo Co., Ltd. (Tokyo, Japan); [bis-(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid] (BAPTA) from Molecular Probes (Eugene, OR); tetraethylammonium chloride (TEA) from Merck (Darmstadt, Germany); guanosine 5'-*O*-(2-thiodiphosphate) (GDP β S) from Boehringer (Mannheim, Germany); acetyl- β -methylcholine chloride (methacholine, MCh), L-glutamate, cadmium chloride (Cd²⁺), barium chloride (Ba²⁺), *N*-methyl-D-glucamine chloride (NMG), atropine, guanosine 5'-triphosphate (GTP), guanosine 5'-*O*-(3-thiotriphosphate) (GTP γ S), and kynurenic acid from Sigma (St. Louis, MO); and pertussis toxin from List Biological Laboratories (Campbell, CA). Pirenzepine was kindly provided by Dr. Karl Thoma (Biberach/Riss, Germany) and

(-)- β -*p*-chlorophenyl-GABA (baclofen) was donated by Ciba-Geigy Ltd. (Basle, Switzerland).

Numerical data in the text and figures are presented as the mean $\pm$ SEM. Student's *t* test was used to compare means when appropriate. Differences between two groups were assessed using the nonparametric Mann-Whitney *U* test. In some experiments, statistical comparisons between groups were made by using ANOVA and Fisher PLSD as post-tests. Differences with *p* < 0.05 were considered significant.

Results

Responses to stimulation of postsynaptic mGluRs and muscarinic receptors were investigated in CA3 pyramidal neurons voltage clamped at a holding potential of -60 mV. Application of 1S,3R-ACPD, a selective mGluR agonist (Irving et al., 1990) as well as methacholine (MCh), a muscarinic agonist, led to an inward current associated with a decrease in membrane conductance corresponding to a reduction in $I_{K,leak}$ (Fig. 1*A,B*, insets), as previously described (Guérineau et al., 1994). Earlier studies have shown that low concentrations of 1S,3R-ACPD and MCh also reduce the potassium currents I_M and I_{AHP} (Charpak et al., 1990; Charpak and Gähwiler, 1991). Under conditions of whole-cell patch-clamp recording, however, these currents are not significantly involved in these responses (Guérineau et al., 1994).

When the concentration of extracellular potassium was elevated above 5 mM, 1S,3R-ACPD (50 μ M for 30 sec) and MCh (5 μ M for 30 sec) induced, in cells voltage clamped at -60 mV, a biphasic response consisting of an inward current followed by a slowly developing outward current (Fig. 1*A,B*). To characterize these currents, most experiments were done at [K⁺]_o = 16 mM and [Na⁺]_o = 124 mM, conditions under which the inward current was maximal. Application of hyperpolarizing voltage steps (10 mV, 800 msec, 0.1 Hz) revealed that the inward current was associated with an increase in membrane conductance (Fig. 1*Ab,Bb*, traces 2), whereas the outward current was accompanied by a decrease in membrane conductance (Fig. 1*Ab,Bb*, traces 3). These changes in membrane conductance are summarized in Table 1.

The induction of this biphasic current by 1S,3R-ACPD (or MCh) was mimicked by application of L-glutamate (250–500 μ M for 30 sec) in the presence of ionotropic glutamate receptor antagonists CNQX (20 μ M) and D-APV (40 μ M) (*n* = 12; Fig. 7*B*). Because of the selectivity of 1S,3R-ACPD for mGluRs, all subsequent experiments were performed with this agonist.

Concentration–response curves for 1S,3R-ACPD-induced inward and outward currents

Repetitive exposure to agonists resulted in 'rundown' of responses, probably due to the loss of cytosolic components, as commonly observed with whole-cell recordings. For this reason, each neuron was tested with not more than three different concentrations of agonists. The concentration–response curves for 1S,3R-ACPD-induced inward and outward currents were well fitted by an asymmetric sigmoidal function, as illustrated in Figure 2. The responses saturated at 50 μ M 1S,3R-ACPD, and exhibited EC₅₀s of 10.8 μ M and 10.4 μ M for the inward and outward currents, respectively. In all of the following experiments, we used the saturating concentrations of 50 μ M for 1S,3R-ACPD and 5 μ M for MCh. The inward and the outward currents were regarded as independent processes because no linear correlation between the amplitude of inward and outward currents from cell to cell was found when both parameters were plotted against each other (data not shown).

The first part of this study deals with the voltage dependence,

Figure 1. 1S,3R-ACPD and MCh induce a biphasic inward/outward current. A CA3 pyramidal cell was clamped at -60 mV in 16 mM extracellular K^+ solution, and hyperpolarizing voltage pulses were periodically applied to monitor membrane conductance. 1S,3R-ACPD ($50 \mu M$, Aa) and MCh ($5 \mu M$, Ba) each induced an inward current followed by a slowly developing outward current. Ab and Bb, The inward current was associated with an increase in membrane conductance (traces a2 and b2), whereas the outward current was associated with a decrease in membrane conductance (traces a3 and b3). Insets, The two agonist-induced inward currents recorded in 2.7 mM external K^+ . These currents were associated with a decrease in membrane conductance.

ionic selectivity, and pharmacology of the inward current. The characteristics of the outward current are described in the second part.

1S,3R-ACPD and MCh activate a nonselective cation-permeable conductance

The reversal potentials of 1S,3R-ACPD- and MCh-induced inward currents were assessed with a voltage ramp protocol (from -120 to -50 mV, 3.5 sec) before (ramp 1) and during the application of the two agonists (ramp 2 evoked at the peak of the current) (Fig. 3). The currents generated during the voltage ramp were well fitted by a computed linear regression ($r^2 = 0.98$ to 1), which were then used to determine the potential at which the currents intersect. According to this protocol, the extrapolated reversal potentials were -9.1 ± 5.8 mV ($n = 18$) and -12.9

± 8.7 mV ($n = 9$) for 1S,3R-ACPD and MCh, respectively ($p > 0.05$). Alternatively, the reversal potential was determined by plotting the current-voltage curve constructed from 1S,3R-ACPD-induced current measured at three different holding potentials (-90 , -50 , and -10 mV, data not shown, $n = 3$). No appreciable differences (≤ 5 mV) were found in the values of the reversal potential calculated by either method. These values are close to the equilibrium potential for monovalent cations, as predicted by the Nernst equation (-1.2 mV at $25^\circ C$) given the experimental $[cations]_i = 144.8$ mM and $[cations]_o = 152$ mM. This result is consistent with the involvement of a nonselective cationic conductance.

Subtraction of control currents from those recorded in the presence of agonists yielded the currents activated by 1S,3R-ACPD or MCh (Fig. 3Ac,Bc) and showed that the conductance underlying this current is voltage independent in the membrane potential range from -120 to -50 mV.

The properties of the 1S,3R-ACPD- and MCh-induced conductance were studied in more detail by modifying the extracellular monovalent cation concentrations. When $[K^+]_o$ was halved (16 to 8 mM), the amplitude of the inward current activated by the two agonists at -60 mV was reduced by 72% for 1S,3R-ACPD ($n = 8$) and 73% for MCh ($n = 6$) ($p < 0.05$, Fig. 4A,C). Decreasing $[Na^+]_o$ from 124 mM to 70 mM (Fig. 4B,C) also led to a decrease in current amplitude (by 34%, $n = 5$ and 31%, $n = 6$ for 1S,3R-ACPD and MCh, respectively, $p < 0.05$).

Table 1. Membrane conductance measured before (control) and at the peak of the inward and the outward currents induced by 1S,3R-ACPD and MCh

	1S,3R-ACPD ($50 \mu M$)	MCh ($5 \mu M$)
Control (nS)	17.4 ± 1.8	19.4 ± 2.0
Inward current (nS)	25.0 ± 2.9	28.7 ± 3.1
Outward current (nS)	12.5 ± 1.8	15.1 ± 1.5
Number of cells	13	12

Figure 2. Concentration–response curves for the 1S,3R-ACPD-induced inward and outward currents. Cells were bathed in 16 mM K^+ -containing external saline and voltage clamped at -60 mV. Points represent the mean $\pm$ SEM of the 1S,3R-ACPD-induced inward (open circles) and outward (filled circles) currents measured at the peak of the response. The curves that fitted to the data were derived from an asymmetric sigmoid equation $I = I_{min} + (I_{max} - I_{min}) / \{1 + ([agonist]/EC_{50})^{-n}\}$ where I is the normalized current amplitude and n is the estimated Hill coefficient (2.0 for the inward current and 4.6 for the outward current). The correlation coefficients are 0.98 and 0.96 for 1S,3R-ACPD-induced inward and outward current, respectively. Five to twenty cells were tested at each agonist concentration.

Moreover, as expected for a response mediated by a change in cationic permeability, decreasing $[K^+]_o$ or $[Na^+]_o$, significantly shifted the reversal potential of 1S,3R-ACPD- and MCh-induced currents to more negative values compared to those found for 16 mM $[K^+]_o$ and 124 mM $[Na^+]_o$ (Table 2, $p < 0.05$).

Outward movement of chloride is unlikely to contribute to this response, since the equilibrium potential for Cl^- ions under the present conditions was determined to be -60.8 mV (at $25^\circ C$). Neither the amplitude of the 1S,3R-ACPD- or MCh-induced responses (138.5 ± 27.1 pA, $n = 5$ for 1S,3R-ACPD and 145.4 ± 24.1 pA, $n = 5$ for MCh, $p > 0.05$), nor their reversal potentials (-8.3 ± 4.4 mV, $n = 5$ for 1S,3R-ACPD and -6.2 ± 3.2 mV, $n = 5$ for MCh, $p > 0.05$) were significantly changed by removing extracellular Ca^{2+} . Taken together, these results indicate that the channel underlying the inward current is primarily permeable to the monovalent cations K^+ and Na^+ .

The permeability ratio $P_{Na}:P_K$ of the inward current was calculated using the Goldman-Hodgkin-Katz constant field equation (Goldman, 1943; Hodgkin and Katz, 1949). For $[K^+]_o$ below 5 mM, $P_{Na}:P_K$ was 0.07. This value is close to the resting basal permeability for these two ions (Kuffler et al., 1984), and may explain why this current was not detectable in 2.7 mM $[K^+]_o$. When the external K^+ concentration was raised to 16 mM, the ratio appeared to be substantially increased to a $P_{Na}:P_K$ of 0.76 for 1S,3R-ACPD- and 1.09 for MCh-induced responses ($n = 23$ and 15 for 1S,3R-ACPD and MCh, respectively).

1S,3R-ACPD and MCh act on the same cationic channels

As illustrated in the previous figures, application of 1S,3R-ACPD or MCh produced seemingly identical responses. This

raises the possibility that the receptors for 1S,3R-ACPD and MCh are coupled to the same channels. To test this hypothesis, the cultures were exposed to a mixture of 1S,3R-ACPD and MCh (Fig. 5). At low concentrations, concomitant application of the two agonists induced a significant increase in the current amplitude in comparison to that observed in response to only one agonist (Fig. 5A, upper traces; $n = 13$). By contrast, no additive effect was found at saturating agonist concentrations (Fig. 5A, lower traces; $n = 7$) suggesting that the same channels mediate the response to both 1S,3R-ACPD and MCh.

Ionic sensitivity of the cationic current

The effects of divalent cations on the amplitude of current recorded at a holding potential of -60 mV in 16 mM $[K^+]_o$ were examined. Extracellular exposure to Ba^{2+} ions (1 mM) decreased the 1S,3R-ACPD- and MCh-induced currents by $60.4 \pm 7.5\%$ and $48.5 \pm 8.4\%$, respectively ($p < 0.05$, $n = 15$; Fig. 6Aa,b). Increasing extracellular Mg^{2+} from 2 to 10 mM also reduced the cationic currents induced by 1S,3R-ACPD and MCh was observed ($82.8 \pm 6.1\%$ and $86.2 \pm 7.3\%$, respectively, $p < 0.05$, $n = 10$; Fig. 6Ba,b). The responses elicited by the two agonists were also reduced by $95.0 \pm 5.0\%$ for 1S,3R-ACPD and $92.4 \pm 6.0\%$ for MCh ($n = 10$) in Cd^{2+} -containing extracellular saline (100 μM). The cationic current did not, however, require an increase in intracellular Ca^{2+} concentration because loading cells with the Ca^{2+} chelator BAPTA (20 mM, 10 min before agonist exposure) did not modify the current ($p > 0.05$, $n = 21$).

1S,3R-ACPD- and MCh-stimulated inward currents were not

Figure 3. 1S,3R-ACPD and MCh activate a nonselective, voltage-independent conductance. *Aa, b,* and *Ba, b,* The reversal potential of the responses to 1S,3R-ACPD and MCh was assessed by linearly increasing the voltage from -120 to -50 mV (3.5 sec) before (*ramp 1*) and during (*ramp 2*) the application of agonists. The extrapolated reversal potential for these currents was close to the equilibrium potential for cations (144.8 mM [cation], and 152 mM [cation]). *Ac* and *Bc,* Subtraction of control currents from those in the presence of 1S,3R-ACPD and MCh revealed that the currents activated by the two agonists varied linearly with membrane potential.

modified in either Cs^+ -loaded cells (140 mM Cs^+ -gluconate in the patch pipette, $n = 15$) or in the presence of extracellular Cs^+ (2 mM, $n = 8$, data not shown, $p > 0.05$). Bath application of tetraethylammonium (TEA, 20 mM), however, greatly reduced the responses to both 1S,3R-ACPD ($73.2 \pm 4.3\%$) and MCh ($85.0 \pm 6.1\%$) ($p < 0.05$, $n = 13$). Lower TEA concentrations (1 mM) had no effect on the cationic current ($n = 5$, data not shown). Pooled data are presented in the histogram (Fig. 6C).

Characterization of glutamatergic and cholinergic receptors mediating 1S,3R-ACPD and MCh-induced cationic current

Although 1S,3R-ACPD is considered to be a selective agonist at mGluRs (Irving et al., 1990), we examined its response in the presence of the NMDA and AMPA/kainate receptor antagonists D-APV (40 μM) and CNQX (20 μM) (Watkins and Olverman, 1987) to definitively exclude the involvement of ionotropic glutamate receptors that are also coupled to cation-permeable channels (Fig. 7A). Neither the responses to 1S,3R-ACPD nor MCh were significantly modified by this treatment (156.7 ± 38.1 pA under control conditions, $n = 6$ versus 135.8 ± 30.0 pA in the presence of antagonists, $n = 6$, $p > 0.05$ for 1S,3R-ACPD and 168.8 ± 26.5 pA, $n = 6$ versus 126.3 ± 37.5 pA, $n = 4$, $p > 0.05$ for MCh). Kynurenic acid (2 mM), a nonselective ionotropic glutamate receptor antagonist, was also without effect ($n = 4$, data not shown). In the presence of bath-applied MCPG (1 mM), a selective and competitive antagonist at mGluR1 and mGluR2 (Hayashi et al., 1994), the amplitude of the current induced by 1S,3R-ACPD was significantly reduced ($164.3 \pm$

21.1 pA under control conditions, $n = 7$ versus 45.0 ± 10.6 pA in MCPG-containing saline, $n = 6$, $p < 0.05$), while the response to MCh was not modified (152.0 ± 28.7 pA, $n = 5$ versus 136.0 ± 31.9 pA, $n = 5$, $p > 0.05$). 1R,3S-ACPD (100 μM), a potent agonist at phospholipase C-coupled mGluRs in striatum (Manzoni et al., 1992), failed to induce a current ($n = 9$, data not shown). Agonist potency profiles were determined to characterize the mGluRs subtypes involved in the cationic current. In the presence of CNQX and D-APV, L-glutamate (500 μM for 30 sec) or quisqualate (0.5 μM for 30 sec), a potent agonist at mGluR1 and mGluR5 subtypes, were more effective at inducing the inward current than 1S,3R-ACPD at 50 μM ($n = 8$ and 10 , respectively; Fig. 7B). By contrast, L-AP4 (200 μM for 30 sec), an agonist at mGluR4, mGluR6 and mGluR7 subtypes (Nakajima et al., 1993; Tanabe et al., 1993; Okamoto et al., 1994) had no effect on membrane current ($n = 12$). This suggests that the activation of the cationic current may be due to the stimulation of mGluR1 and/or mGluR5 subtypes.

Atropine (1 μM) abolished MCh-induced inward current, indicating an action at muscarinic receptors ($n = 3$; Fig. 7A). The blockade was not reversible after extensive washing (10 – 15 min). In these three cells, the response to 1S,3R-ACPD was not altered by atropine. To characterize in more detail the subtype of muscarinic receptors, we tested the effects of bath-applied pirenzepine, an M_1 receptor antagonist (North et al., 1985). Within 5 min of superfusion with a high concentration of pirenzepine (1 μM), the MCh-induced current was reduced by only $41.5 \pm 10.8\%$ ($n = 4$; Fig. 7A), suggesting that the effect of MCh is probably not due to the activation of M_1 receptors.

Figure 4. The inward current induced by 1S,3R-ACPD or MCh is mainly carried by K^+ and Na^+ ions. Reducing $[K^+]_o$ (Aa and b) or $[Na^+]_o$ (Ba and b) led to a decrease in the amplitude of the 1S,3R-ACPD-induced current in four different cells voltage clamped at -60 mV. C, The effects of changing $[K^+]_o$ and $[Na^+]_o$ on the amplitude of the inward current elicited by the two agonists are summarized in the histogram. The number of recorded cells is indicated in parentheses.

1S,3R-ACPD- and MCh-induced outward current is due to a reduction of a voltage-independent K^+ conductance

In 16 mM K^+ -containing solution at a holding potential of -60 mV, the 1S,3R-ACPD- and MCh-induced cationic current was followed by a slowly developing, long-lasting outward current

Table 2. Mean reversal potentials for 1S,3R-ACPD- and MCh-induced inward current recorded in three different extracellular K^+ or Na^+ concentrations

		E_{rev} (mV) 1S,3R-ACPD	E_{rev} (mV) MCh
$[K^+]_o$ (mM; 124 mM $[Na^+]_o$)	5	-59.7 ± 4.1 (3)	-60.7 ± 2.3 (4)
	8	-51.2 ± 1.8 (9)	-51.2 ± 2.6 (12)
	16	-9.1 ± 4.4 (23)	-3.3 ± 6.2 (15)
$[Na^+]_o$ (mM; 16 mM $[K^+]_o$)	50	-36.0 ± 2.2 (4)	-46.0 ± 1.1 (4)
	70	-31.5 ± 1.7 (4)	-35.0 ± 3.1 (4)
	124	-9.1 ± 4.4 (23)	-3.3 ± 6.2 (15)

These reversal potentials were calculated by computed linear regression of the currents generated during a voltage ramp protocol (-80 to -60 mV, 2 sec). The number of cells tested for each cation concentration is indicated in parentheses.

that was associated with a decrease in membrane conductance (Fig. 8Aa,Ba). To study the voltage dependence of the currents, voltage jumps of 100 msec duration to test potentials ranging from -120 to -30 mV were applied to cells before and during application of 1S,3R-ACPD and MCh (Fig. 8Ab,Bb). The currents suppressed by the two agonists varied linearly with membrane potential and reversed at -53.2 ± 2.7 mV for 1S,3R-ACPD ($n = 3$) and -52.8 ± 2.8 mV for MCh ($n = 3$). These values are close to the equilibrium potential for K^+ , as predicted by the Nernst equation (-57 mV at 25°C , given the experimental $[K^+]_o = 16$ mM and $[K^+]_i = 150$ mM). Similar values were obtained when the reversal potential was determined using the ramp protocol described for Table 2 (-55.1 ± 1.1 mV for 1S,3R-ACPD, $n = 17$ and -56.4 ± 1.5 mV for MCh, $n = 11$, data not shown). The ionic selectivity for K^+ was shown by plotting the reversal potential at three different extracellular K^+ concentrations (Fig. 8Ac,Bc). Lowering $[K^+]_o$ to 2.7 mM reversed the sign of this current, further suggesting that the response corresponds to the decrease in $I_{K,leak}$ previously described (Guérineau et al., 1994).

This K^+ current, which is reduced by 1S,3R-ACPD and MCh, displayed the same sensitivity to receptor antagonists as did the cationic current. MCPG (1 mM) was an effective blocker ($\sim 70\%$) of 1S,3R-ACPD-induced outward current (25.3 ± 15.2

Figure 5. Additive effects of 1S,3R-ACPD and MCh on the cationic conductance. *A*, Cells were held at -60 mV in 16 mM external K^+ solution and exposed to 1S,3R-ACPD or MCh alone or together. At low concentrations, responses induced by concomitant application of 1S,3R-ACPD and MCh were additive (*upper traces*). At saturating concentrations, the response evoked by combined application of both drugs was not larger than that evoked by either drug alone (*lower traces*). Pooled data are presented in the histogram (*B*). The number of tested cells is indicated in parentheses. *, $p < 0.05$, as compared to control values.

pA, $n = 7$ in the presence of MCPG versus 83.7 ± 22.5 pA, $n = 10$ in control saline, $p < 0.05$) (data not shown). The response to MCh was completely antagonized by atropine ($1 \mu M$, $n = 3$) but only reduced by 45% in pirenzepine-containing saline ($1 \mu M$, 55.7 ± 17.3 pA, $n = 4$ versus 100.3 ± 8.9 pA, $n = 9$, $p < 0.05$) (data not shown). In addition, 1R,3S-ACPD ($100 \mu M$) was ineffective in inducing a response ($n = 9$).

Role of G-proteins

To establish an involvement of G-proteins in the intracellular transduction pathways stimulated by 1S,3R-ACPD and MCh, cells were loaded with GTP γ S (250 – $500 \mu M$ for 10 min), a nonhydrolyzable analog of GTP that irreversibly activates G-proteins (Gilman, 1984). The first application of 1S,3R-ACPD activated the inward cationic current followed by the outward K^+ current, which then remained irreversibly activated (Fig. 9*A*, upper trace), demonstrating the involvement of G-proteins. In contrast, the cationic currents were not affected by GTP γ S loading, as shown by successive applications of 1S,3R-ACPD or MCh in the same cell ($n = 19$; Fig. 9*A*). The cationic current induced by 1S,3R-ACPD or MCh was also not modified in GDP β S-loaded cells (250 – $500 \mu M$ for 10 min), a nonhydrolyzable analog of GDP that prevents G-protein activation (Eckstein et al., 1979). We assumed that GDP β S had effectively diffused into cells if the outward current was decreased by more than

70%. As summarized in panel 9*B*, the cationic current thus appears to be insensitive to G-protein activation or inactivation, whereas the outward current is respectively increased or abolished by these manipulations. Further evidence for the involvement of different transduction mechanisms mediating the cationic current versus $I_{K,leak}$ was obtained by measuring the onset latencies for each response. The onset latency for the inward current recorded in 16 mM $[K^+]_o$ was significantly lower than that measured in 2.7 mM $[K^+]_o$ (16.5 ± 0.8 sec versus 28.9 ± 2.5 sec for 1S,3R-ACPD, $n = 19$ and 10 respectively, $p < 0.05$, and 16.9 ± 0.9 sec versus 25.3 ± 1.4 sec for MCh, $n = 21$ and 13 , respectively, $p < 0.05$) (see also Fig. 1).

In a further attempt to characterize the possible involvement of G-proteins, cells were treated with pertussis toxin (500 ng/ml for 48 hr), which inactivates G-proteins of the G_i/o subtype (Kataoka and Ui, 1982). Responses to baclofen were now reduced by more than 80% ($n = 10$, $p < 0.05$), as expected for an intracellular transduction pathway known to be mediated by G-proteins sensitive to pertussis toxin (Andrade et al., 1986). In these cells, however, the inward cationic current or the outward K^+ current induced by 1S,3R-ACPD or MCh (data not shown, $n = 8$ and 10 for 1S,3R-ACPD and MCh, respectively, $p > 0.05$) were not modified.

Involvement of a second messenger

Since no evidence could be obtained to show an involvement of G-proteins in mediating the cationic current, this raises the question whether metabotropic, that is, second messenger, pathways underlie this response. To resolve this point, the effects of 1S,3R-ACPD and MCh on single-channel activity were investigated. A cationic channel was identified in 13 patches out of 136 cells (9.6%) ($n = 5$ for 1S,3R-ACPD and 8 for MCh). Figure 10*A* illustrates a typical recording of this channel. Bath application of MCh ($100 \mu M$) outside the recording pipette to a cell voltage clamped at the resting membrane potential triggered, after several seconds latency, inward currents, reflecting ion flux through the open state of the channel. To identify the ionic specificity of this channel, the reversal potential was calculated by plotting the amplitude of the unitary current recorded at different potentials (Fig. 10*B*). The data were fitted with a linear regression, giving a reversal potential of $+48.9$ mV relative to resting membrane potential. Assuming a resting potential of -50 mV, this would correspond to a reversal potential close to 0 mV, consistent with the value determined for the whole-cell recordings (Fig. 3, Table 2). The slope conductance for the channel depicted in Figure 10 was 14.3 pS. The data for all the recorded channels are summarized in Table 3. In further experiments, unitary recordings from voltage-gated potassium channels were obtained in 16 mM $[K^+]_o$ (data not shown). These currents reversed at a potential of -5.8 ± 4.5 mV relative to resting potential ($n = 5$), corresponding to a membrane potential of ~ -55 mV. This is close to the equilibrium potential of -57.5 mV for K^+ ions calculated with the Nernst equation. Thus, these channels could easily be distinguished from cationic channels.

Discussion

Stimulation of mGluRs and cholinergic muscarinic receptors was shown to activate a nonselective (mainly K^+/Na^+) cationic conductance in CA3 hippocampal neurons in the presence of elevated extracellular K^+ concentration (≥ 5 mM). This initial conductance increase was followed by a second response due to the previously described reduction of $I_{K,leak}$ (Guérineau et al.,

Figure 6. Ionic sensitivity of the cationic conductance increase. Cells were bathed in 16 mM K⁺-containing external saline and voltage clamped at -60 mV. Bath application of divalent cations, Ba²⁺ (1 mM, *Aa* and *b*) and Mg²⁺ (10 mM, *Ba* and *b*) reversibly diminished the 1S,3R-ACPD- and MCh-induced cationic currents in the same cells. *C*, Summary of the effects of different divalent cations (Ba²⁺, Mg²⁺, Cd²⁺ 100 μ M), K⁺ channel inhibitors (TEA 20 mM, Cs⁺ 140 mM in the patch pipette) and BAPTA (20 mM) on the response induced by the two agonists. The number of tested cells is indicated in parentheses. *, $p < 0.05$, as compared to control values.

1994). These effects of 1S,3R-ACPD and MCh are most likely mediated by a direct activation of postsynaptic receptors, since all experiments were performed in the presence of TTX. Furthermore, responses were not altered following 5–15 min of superfusion with Ca²⁺-depleted solution.

The cationic conductance increase

The inward current amplitude as well as its reversal potential were modified by changing the extracellular K⁺ and Na⁺ concentrations, consistent with the activation of a nonselective cation channel that is permeable to both K⁺ and Na⁺ ions. Previous studies have reported the presence of a slow afterdepolarization (sADP) in CNS neurons, which is potentiated by mGluR agonists (Greene et al., 1992; Constanti and Libri, 1992; Zheng and Gallagher, 1992; Caesar et al., 1993) and by muscarinic agonists (Schwindt et al., 1988; Caesar et al., 1993). I_{ADP} has been characterized as a nonspecific cation current that is activated by Ca²⁺ (Crépel et al., 1994). The cationic current that we observe does not, however, correspond to I_{ADP} because intracellular BAPTA did not affect the current. Another Na⁺/K⁺ current described in hippocampal cells, I_{O} (or I_{H}) is activated by hyperpolarization to membrane potentials negative to ~ -70 mV, and is sensitive to

extracellular Cs⁺ but insensitive to Ba²⁺ (Brown and DiFrancesco, 1980; Halliwell and Adams, 1982; Colino and Halliwell, 1993). Unlike I_{O} , however, the cationic current activated by 1S,3R-ACPD and MCh persists in the presence of extracellular Cs⁺, and is greatly reduced in the presence of Ba²⁺. A similar voltage-insensitive cationic current has been found to contribute to the depolarizing response to muscarine and substance P in the locus ceruleus (Shen and North, 1992a,b; Koyano et al., 1993).

The amplitude of 1S,3R-ACPD- and MCh-activated cationic current was reversibly decreased by extracellular application of divalent cations. This could be explained by the presence of an external divalent cation-binding site in the pore of the channel (Hille, 1992). A similar mechanism may account for the observed blockade by TEA of 1S,3R-ACPD- and MCh-induced currents in hippocampus, and muscarine-induced current in the locus ceruleus (Shen and North, 1992a). However, the cationic current we report here is somewhat different from the current described by Shen and North, in that low TEA concentrations (1 mM) did not modify its amplitude. Studies of a muscarinic receptor-activated cation channel in guinea-pig ileum indicate that TEA does not modify receptor binding by muscarinic ago-

Figure 7. Characterization of receptors mediating the 1S,3R-ACPD- and MCh-induced cationic currents. **A**, The inward current induced by 1S,3R-ACPD was reversibly reduced in the presence of extracellular MCPG (1 mM), but was unaffected by CNQX (20 μ M) and D-APV (40 μ M). Atropine (1 μ M) fully antagonized the response to MCh, while pirenzepine at high concentration (1 μ M) inhibited the cationic current by only 40%. **B**, L-glutamate (500 μ M) and quisqualate (0.5 μ M), a potent agonist at mGluR1 and mGluR5 subtypes, induced the cationic current in the presence of CNQX (20 μ M) and D-APV (40 μ M). L-AP4 (200 μ M), a potent agonist at mGluR4 and mGluR6 subtypes, failed to activate a membrane current. The number of recorded cells is indicated in parentheses. *, $p < 0.05$, as compared to control values.

nists but acts downstream by occluding the channel (Chen et al., 1993). Large positively charged molecules such as TEA may be driven into the channel mouth or attracted to a negatively charged binding site located midway down the channel pore and thus interfere with the permeation of cations (Hille, 1992). On the other hand, the amplitude of the currents was not significantly changed by intra- or extracellular Cs^+ , indicating that the channel is permeable to small cations such as Cs^+ . In contrast to the cationic conductance described in the cortex (Andrade, 1993) and in area CA1 of the hippocampus (Colino and Halliwell, 1993), the response we observed was not Ca^{2+} dependent.

The permeability ratio $P_{\text{Na}}/P_{\text{K}}$ was calculated to be 0.07 for $[\text{K}^+]_o \leq 5$ mM but was greatly increased upon raising $[\text{K}^+]_o$ to 16 mM. This behavior is not compatible with a channel model based on the assumption of independent membrane fluxes of ions. Rather, an interaction between the two cationic species may be occurring within the channel to account for the nonlinearity of the relationship between extracellular K^+ concentration and channel permeability (Hille, 1992).

Characterization of receptors

The action of 1S,3R-ACPD is clearly mediated by mGluRs. Specific antagonists at ionotropic glutamate receptors did not alter the inward current activated by 1S,3R-ACPD, whereas MCPG, a selective and competitive antagonist at mGluR1 and mGluR2

(Hayashi et al., 1994), was an effective blocker of the 1S,3R-ACPD-induced inward current but not of the MCh-induced response. L-AP4, the most potent known agonist at mGluR4, mGluR6, and mGluR7 (Nakajima et al., 1993; Tanabe et al., 1993; Okamoto et al., 1994), was without effect, whereas quisqualate, a potent agonist at mGluR1 and mGluR5, induced responses comparable but greater than those observed with 1S,3R-ACPD. Based on these pharmacological data, and on *in situ* hybridization studies that demonstrate that hippocampal CA3 pyramidal cells express mainly mGluR1 and mGluR5 (Abe et al., 1992; Shigemoto et al., 1992), the mGluRs mediating the observed responses are likely to correspond to these subtypes.

Pirenzepine (1 μ M), which has a K_D of 10 nM at the M_1 subtype of the muscarinic receptor (North et al., 1985), inhibited only 40% of the cationic current. Thus, the MCh-induced current is probably mediated by receptors other than the M_1 subtype. Previous studies describing the activation of a cationic conductance by cholinergic agonists have reported the involvement of the M_2 subtype muscarinic receptor in locus ceruleus neurons (Egan and North, 1985) and in hippocampal CA1 cells (Colino and Halliwell, 1993).

Pharmacologically indistinguishable mGluRs and muscarinic receptors gate both the increase in cation conductance and the decrease in K^+ conductance. Both conductances were sensitive to the same pharmacological agents, that is, blockade by MCPG

Figure 8. A reduction of $I_{K,leak}$ by 1S,3R-ACPD and MCh is responsible for the outward current. *Aa* and *Ba*, 1S,3R-ACPD- and MCh-induced typical biphasic responses in a cell voltage clamped at -60 mV in 16 mM $[K^+]_o$. *Ab* and *Bb*, Steady-state I/V curves for the two agonist-evoked outward currents were determined by applying voltage steps (100 msec) to test potentials ranging from -120 to -30 mV in the same cell. Subtraction of control currents from those during 1S,3R-ACPD and MCh revealed that the currents suppressed by the two agonists varied linearly with membrane potential and that the outward current reversed at a potential close to the equilibrium potential for K^+ ions. *Ac* and *Bc*, Plots of pooled data for the reversal potential at three different $[K^+]_o$. The solid line represents the relationship of reversal potential to $[K^+]_o$, as predicted by the Nernst equation. The number of tested cells is indicated in parentheses.

for 1S,3R-ACPD-induced currents and sensitivity to atropine but not to pirenzepine for MCh-activated current. This raises the possibility that the two currents result from activation of the same glutamatergic and cholinergic muscarinic receptors, each receptor coupling to two different types of ion channels. The fact that the EC_{50} s for the two currents are in the same range (~ 10 μ M for 1S,3R-ACPD) argues in favor of this hypothesis. The availability of receptor subtype-specific agonists and antagonists will permit the resolution of this point in the future.

The delayed decrease in $I_{K,leak}$

In addition to activation of a nonselective cationic conductance, 1S,3R-ACPD and MCh caused a reduction of a K^+ conductance. According to its electrophysiological and pharmacological characteristics, that is, time course, reversal potential, voltage independence, blockade by the selective mGluRs antagonist MCPG, and similar sensitivity to intracellular loading with guanine nucleotides, this response corresponds to the previously characterized decrease in $I_{K,leak}$ (Guérineau et al., 1994).

Mechanisms of receptor-effector coupling

Our results suggest that mGluRs and muscarinic receptors gate a common cationic conductance. The inward currents induced by 1S,3R-ACPD and MCh displayed a similar time course, reversal potential, and current-voltage relationship. Furthermore, the responses induced by both agonists were additive at submaximal concentrations but mutually occlusive at saturating concentrations. Pretreatment with pertussis toxin did not modify either response and, surprisingly, neither did intracellular loading with GDP β S or GTP γ S. In the same cells, these guanine nucleotide analogs markedly altered the outward current corresponding to the reduction in a previously characterized leak K^+ current (Guérineau et al., 1994). Furthermore, the shorter onset latency

observed for the cationic current versus $I_{K,leak}$ is consistent with the activation of two separate intracellular transduction pathways. We conclude that either the cationic current is activated by a novel glutamate receptor not coupled to G-proteins, that guanine nucleotides introduced into cells do not have access to the receptor/G-protein complexes, perhaps due their localization on distal dendrites, or that a novel as yet uncharacterized molecule couples these receptors to the cationic channels. We have previously observed that responses to stimulation of GABA $_B$ receptors, which are localized on hippocampal pyramidal cell dendrites (Newberry and Nicoll, 1985), are sensitive to GDP β S and GTP γ S, indicating effective diffusion from the pipette into at least part of the dendritic tree (Guérineau et al., 1994). Although our single-channel experiments show that cationic channels can be found on the cell body, we cannot at present rule out a localization of the receptors on distal dendrites.

Is "metabotropic" an appropriate label for a non-G-protein-coupled receptor? To answer this question, one must first rule out the involvement of an ionotropic receptor. This was the rationale for our single-channel experiments. The fact that responses were observed in cell-attached patches when agonists were applied outside the recording pipette implicates diffusible second messengers in coupling these receptors to the cationic channels (Hille, 1994). Furthermore, the long latency between agonist application and channel activation suggests that second-messenger production occurs at a distance from the channels. Therefore, although the receptor does not appear to be G-protein-coupled, the term metabotropic is, nonetheless, justified. Similar currents that appear to be G-protein-independent have been described for a number of receptor systems (Olesen et al., 1988; Koyano et al., 1993; Twitchell and Rane, 1993; White et al., 1993) including mGluRs (Kehoe, 1994).

Figure 9. Role of G-proteins in the mediation of 1S,3R-ACPD- and MCh-induced currents. **A**, In GTP γ S-loaded cells (250 μ M for 10 min), the first application of 1S,3R-ACPD (upper trace) or MCh (lower trace) induced the inward current followed by the prolonged activation of the outward current. Subsequent exposures to 1S,3R-ACPD or MCh, in the same cells, activated only the inward current. **B**, Histograms summarizing the effects of GTP (100 μ M), GTP γ S (250–500 μ M) and GDP β S (500 μ M) on the amplitude of both inward cationic and outward K⁺ currents induced by 1S,3R-ACPD and MCh. The cationic current remained insensitive to these manipulations, while the inhibition of K⁺ current was enhanced by GTP or GTP γ S, and was strongly reduced by GDP β S loading. The number of recorded cells is indicated in parentheses. *, $p < 0.05$, as compared to control values.

Figure 10. Single-channel recording of cationic channel activity. Unitary currents were recorded in the cell-attached configuration at resting potential (RP) with a patch pipette filled with extracellular saline containing 16 mM K⁺ and 0.5 μ M TTX. **A**, Bath application of MCh (100 μ M) outside the pipette triggered inward currents reflecting ion flux through the open state of the channel. **B**, The reversal potential of the unitary current was assessed by plotting the amplitude of the current measured at different pipette potentials. The data were fitted by a linear regression ($r^2 = 0.98$), giving a reversal potential close to +50 mV relative to RP and a slope conductance of 14.3 pS.

Table 3. Characteristics of cationic channels opened by prolonged bath-application of 1S,3R-ACPD or MCh

	1S,3R-ACPD (500 μ M)	MCh (100 μ M)
Latency (sec)	38.2 $\pm$ 10.7 (5)	21.8 $\pm$ 8.3 (6)
Amplitude at RP (pA)	0.97 $\pm$ 0.04 (5)	0.82 $\pm$ 0.07 (8)
Unitary conductance (pS)	17.3 $\pm$ 4.2 (3)	17.5 $\pm$ 0.9 (6)
E_{rev} , relative to RP (mV)	+42.9 $\pm$ 4.9 (3)	+57.4 $\pm$ 3.7 (6)

Currents were recorded in the cell-attached configuration with a patch pipette filled with an external saline containing 16 mM K^+ and 0.5 μ M TTX. For each parameter, no statistical difference between 1S,3R-ACPD- or MCh-induced unitary cationic currents was found. RP denotes resting potential. The number of recorded cells is indicated in parentheses.

Physiological implications

These results extend previous observations on the remarkable similarities in the responses to stimulation of mGluRs and muscarinic acetylcholine receptors (Charpak et al., 1990; Miller, 1991). Activation of these receptors modulates several potassium currents, including I_M , I_{AHP} , and $I_{K, leak}$, calcium currents, and the calcium-dependent cationic current underlying I_{ADP} (see Nicoll et al., 1990; Miller, 1991; Gerber and Gähwiler, 1994). The fact that two separate neurotransmitter systems activate receptors mediating slow modulatory responses in parallel attests to the physiological importance of the underlying currents. For example, activation of either of these receptor systems has been shown to facilitate the induction of long-term potentiation (Blitzer et al., 1990; Katsuki et al., 1992; Bashir et al., 1993). The glutamatergic system may be primarily involved for local modulation while cholinergic pathways may represent a mechanism for communication between separate brain areas (e.g., septohippocampal).

The cationic current was only observed when the extracellular K^+ concentration was raised above 5 mM. Although the resting extracellular K^+ concentration in brain lies between 2.8 to 3.4 mM, increases to as much as 10–15 mM are seen during epileptic seizures, after repetitive stimulation or following exposure to excitatory amino acids, whereas concentration as high as 100 mM have been observed during ischemia (see Walz and Hertz, 1983).

Activation of a nonspecific Na^+/K^+ conductance leads to a membrane depolarization that could contribute to the excitatory effects of glutamate and acetylcholine (McCormick and Prince, 1986; Benson et al., 1988). Since the cationic conductance becomes more prominent upon accumulation of extracellular K^+ ions, it may participate in positive feedback mechanisms important in physiological processes such as synaptic plasticity or in pathological situations such as epilepsy.

References

- Abe T, Sugihara H, Nawa H, Shigemoto R, Mizuno N, Nakanishi S (1992) Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/ Ca^{2+} signal transduction. *J Biol Chem* 267:13361–13368.
- Andrade R (1993) Muscarinic receptors induce the appearance of a calcium-activated cation conductance in rat cortex. *Soc Neurosci Abstr* 19:117.11.
- Andrade R, Malenka RC, Nicoll RA (1986) A G-protein couples serotonin and GABA_B receptors to the same channel in the hippocampus. *Science* 234:1261–1265.
- Bashir ZI, Bortolotto ZA, Davies CH, Beretta N, Irving AJ, Seal AJ, Henley JM, Jane DE, Watkins JC, Collingridge GL (1993) Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* 363:347–350.
- Baskys A, Bernstein NK, Barolet AW, Carlen PL (1990) NMDA and quisqualate reduce a Ca-dependent K^+ current by a protein kinase-mediated mechanism. *Neurosci Lett* 112:76–81.
- Benson DM, Blitzer RD, Landau EM (1988) An analysis of the depolarization produced in guinea-pig hippocampus by cholinergic receptor stimulation. *J Physiol (Lond)* 404:479–496.
- Blitzer RD, Gil O, Landau EM (1990) Cholinergic stimulation enhances long-term potentiation in the CA1 region of rat hippocampus. *Neurosci Lett* 119:207–210.
- Brown H, DiFrancesco D (1980) Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J Physiol (Lond)* 308:331–351.
- Caeser M, Brown DA, Gähwiler BH, Knöpfel T (1993) Characterization of a calcium-dependent current generating a slow afterdepolarization of CA3 pyramidal cells in rat hippocampal slice cultures. *Eur J Neurosci* 5:560–569.
- Charpak S, Gähwiler BH (1991) Glutamate mediates a slow synaptic response in hippocampal slice cultures. *Proc R Soc Lond [Biol]* 243:221–226.
- Charpak S, Gähwiler BH, Do KQ, Knöpfel T (1990) Potassium conductances in hippocampal neurons blocked by excitatory amino-acid transmitters. *Nature* 347:765–767.
- Chen S, Inoue R, Ito Y (1993) Pharmacological characterization of muscarinic receptor-activated cation channels in guinea-pig ileum. *Br J Pharmacol* 109:793–801.
- Colino A, Halliwell JV (1993) Carbachol potentiates Q current and activates a calcium-dependent non-specific conductance in rat hippocampus *in vitro*. *Eur J Neurosci* 5:1198–1209.
- Constanti A, Libri V (1992) Trans-ACPD induces a slow post-stimulus inward tail current (I_{ADP}) in guinea-pig olfactory cortex neurones *in vitro*. *Eur J Pharmacol* 214:105–106.
- Crépel V, Aniksztejn L, Ben-Ari Y, Hammond C (1994) Glutamate metabotropic receptors increase a Ca^{2+} -activated nonspecific cationic current in CA1 hippocampal neurons. *J Neurophysiol* 72:1561–1569.
- Desai MA, Conn PJ (1991) Excitatory effects of ACPD receptor activation in the hippocampus are mediated by direct effects on pyramidal cells and blockade of synaptic inhibition. *J Neurophysiol* 66:40–52.
- Dodd J, Dingleline R, Kelly JS (1981) The excitatory action of acetylcholine on hippocampal neurons of the guinea-pig and rat maintained *in vitro*. *Brain Res* 207:109–127.
- Eckstein F, Cassel D, Levkovitz H, Lowe M, Selinger Z (1979) Guanosine 5'-O-(2-thiodiphosphate): an inhibitor of adenylate cyclase stimulated by guanine nucleotide and fluoride ions. *J Biol Chem* 254:9829–9834.
- Egan TM, North RA (1985) Acetylcholine acts on m₂-muscarinic receptors to excite rat locus coeruleus neurones. *Br J Pharmacol* 85:733–735.
- Gähwiler BH (1981) Organotypic monolayer cultures of nervous tissue. *J Neurosci Methods* 4:329–342.
- Gerber U, Gähwiler BH (1994) Modulation of ionic currents by metabotropic glutamate receptors in the CNS. In: *The metabotropic glutamate receptors* (Conn PJ, Patel J, eds), pp 125–146. Totowa, NJ: Humana.
- Gilman AG (1984) G-Proteins and dual control of adenylate cyclase. *Cell* 36:577–579.
- Goldman DE (1943) Potential, impedance, and rectification in membrane. *J Gen Physiol* 27:37–60.
- Greene C, Schwandt P, Crill W (1992) Metabotropic receptor mediated after-depolarization in neocortical neurons. *Eur J Pharmacol (Mol Pharmacol)* 226:279–280.
- Guérineau NC, Gähwiler BH, Gerber U (1994) Reduction of resting K^+ current by metabotropic glutamate and muscarinic receptors in rat CA3 cells: mediation by G-proteins. *J Physiol (Lond)* 474:27–33.
- Halliwell JV, Adams PR (1982) Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Res* 250:71–92.
- Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 391:85–100.
- Hayashi Y, Sekiyama N, Nakanishi S, Jane DE, Sunter DC, Birse EF, Udvarhelyi PM, Watkins JC (1994) Analysis of agonist and antag-

- onist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. *J Neurosci* 14:3370–3377.
- Hille B (1992) Ionic channels of excitable membranes. Sunderland, MA: Sinauer.
- Hille B (1994) Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci* 17:531–536.
- Hodgkin AL, Katz B (1949) The effect of sodium ions on the electrical activity of the giant axon of the squid. *J Physiol (Lond)* 108:33–77.
- Irving AJ, Schofield JG, Watkins JC, Sunter DC, Collingridge GL (1990) 1S,3R-ACPD stimulates and L-AP3 blocks Ca^{2+} mobilization in rat cerebellar neurons. *Eur J Pharmacol* 186:363–365.
- Jones SW (1985) Muscarinic and peptidergic excitation of bullfrog sympathetic neurones. *J Physiol (Lond)* 366:63–87.
- Katada T, Ui M (1982) Direct modulation of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. *Proc Natl Acad Sci USA* 79:3129–3133.
- Katsuki H, Saito H, Satoh M (1992) The involvement of muscarinic, β -adrenergic and metabotropic glutamate receptors in long-term potentiation in the fimbria-CA3 pathway of the hippocampus. *Neurosci Lett* 142:249–252.
- Kehoe J (1994) Glutamate activated a K^{+} conductance increase in *Aplysia* neurons that appears to be independent of G-proteins. *Neuron* 13:691–702.
- Koyano K, Velimirovic BM, Grigg JJ, Nakajima S, Nakajima Y (1993) Two signal transduction mechanisms of substance P-induced depolarization in locus coeruleus neurons. *Eur J Neurosci* 5:1189–1197.
- Kuffler SW, Nicholls JG, Martin AR (1984) From neuron to brain. Sunderland, MA: Sinauer.
- Lancaster B, Adams PR (1986) Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons. *J Neurophysiol* 55:1268–1282.
- Madison DV, Lancaster B, Nicoll RA (1987) Voltage-clamp analysis of cholinergic action in the hippocampus. *J Neurosci* 7:733–741.
- Manzoni O, Prezeau L, Rassendren FA, Sladeczek F, Curry K, Bockaert J (1992) Both enantiomers of 1-aminocyclopentyl-1,3-dicarboxylate are full agonists of metabotropic glutamate receptors coupled to phospholipase C. *Mol Pharmacol* 42:322–327.
- McCormick DA, Prince DA (1985) Two types of muscarinic response to acetylcholine in mammalian cortical neurons. *Proc Natl Acad Sci USA* 82:6344–6348.
- McCormick DA, Prince DA (1986) Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex *in vitro*. *J Physiol (Lond)* 375:169–194.
- McCormick DA, von Krosigk M (1992) Corticothalamic activation modulates thalamic firing through glutamate “metabotropic” receptors. *Proc Natl Acad Sci USA* 89:2774–2778.
- Miller RJ (1991) Metabotropic excitatory amino acid receptors reveals their true colors. *Trends Pharmacol Sci* 14:365–367.
- Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N, Nakanishi S (1993) Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J Biol Chem* 268:11868–11873.
- Newberry NR, Nicoll RA (1985) Comparison of the action of baclofen with γ -aminobutyric acid on rat hippocampal pyramidal cells *in vitro*. *J Physiol (Lond)* 360:161–185.
- Nicoll RA, Malenka RC, Kauer JA (1990) Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. *Physiol Rev* 70:513–565.
- North RA, Slack BE, Surprenant A (1985) Muscarinic M_1 and M_2 receptors mediate depolarization and presynaptic inhibition in guinea-pig enteric nervous system. *J Physiol (Lond)* 368:435–452.
- Okamoto N, Hori S, Akazawa C, Hayashi Y, Shigemoto R, Mizuno N, Nakanishi S (1994) Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. *J Biol Chem* 269:1231–1236.
- Olesen SP, Davies PF, Clapham DE (1988) Muscarinic-activated K^{+} current in bovine aortic endothelial cells. *Circ Res* 62:1059–1064.
- Schwindt PC, Spain WJ, Foehring RC, Chubb MC, Crill WE (1988) Slow conductances in neurons from cat sensorimotor cortex *in vitro* and their role in slow excitability changes. *J Neurophysiol* 59:450–467.
- Segal M (1982) Multiple actions of acetylcholine at a muscarinic receptor studied in the rat hippocampal slice. *Brain Res* 246:77–87.
- Shen KZ, North RA (1992a) Muscarine increases cation conductance and decreases potassium conductance in rat locus coeruleus neurones. *J Physiol (Lond)* 455:471–485.
- Shen KZ, North RA (1992b) Substance P opens cation channels and closes potassium channels in rat locus coeruleus neurons. *Neuroscience* 50:345–353.
- Shigemoto R, Nakanishi S, Mizuno N (1992) Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an *in situ* hybridization study in adult and developing rat. *J Comp Neurol* 322:121–135.
- Staub C, Vranesic I, Knöpfel T (1992) Response to metabotropic glutamate receptor activation in cerebellar Purkinje cells: induction of an inward current. *Eur J Neurosci* 4:832–839.
- Stratton KR, Worley PF, Baraban JM (1989) Excitation of hippocampal neurons by stimulation of glutamate Qp receptors. *Eur J Pharmacol* 173:235–237.
- Stratton KR, Worley PF, Baraban JM (1990) Pharmacological characterization of phosphoinositide-linked glutamate receptor excitation of hippocampal neurons. *Eur J Pharmacol* 186:357–361.
- Tanabe Y, Nomura A, Masu M, Shigemoto R, Mizuno N, Nakanishi S (1993) Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *J Neurosci* 13:1372–1378.
- Twitchell W, Rane S (1993) A μ -opioid receptor modulates BK current in chromaffin cells by a GTP-independent mechanism. *Soc Neurosci Abstr* 19:294.11.
- Walz W, Hertz L (1983) Functional interactions between neurons and astrocytes. II. Potassium homeostasis at the cellular level. *Prog Neurobiol* 20:133–183.
- Watkins JC, Olverman HJ (1987) Agonists and antagonists for excitatory amino acid receptors. *Trends Neurosci* 10:265–272.
- White RE, Lee AB, Shcherbatko AD, Lincoln TM, Schonbrunn A, Armstrong DL (1993) Potassium channel stimulation by natriuretic peptides through cGMP-dependent dephosphorylation. *Nature* 361:263–266.
- Zheng F, Gallagher JP (1992) Metabotropic glutamate receptor agonists potentiate a slow afterdepolarization in CNS neurons. *Neuroreport* 3:622–624.