Modulation of Multiple Potassium Currents by Metabotropic Glutamate Receptors in Neurons of the Hypothalamic Supraoptic Nucleus

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Schrader, L. A. and J. G. Tasker. Modulation of multiple potassium currents by metabotropic glutamate receptors in neurons of the hypothalamic supraoptic nucleus. J. Neurophysiol. 78: 3428–3437, 1997. We studied the effects of activation of the metabotropic glutamate receptors on intrinsic currents of magnocellular neurons of the supraoptic nucleus (SON) with whole cell patch-clamp and conventional intracellular recordings in coronal slices (400 μm) of the rat hypothalamus. Trans-(z)-1-amino-1,3-cyclopentane dicarboxylic acid (trans-ACPD, 10–100 μM), a broad-spectrum metabotropic glutamate receptor agonist, evoked an inward current (18.7 ± 3.45 pA) or a slow depolarization (7.35 ± 4.73 mV) and a 10–30% decrease in whole cell conductance in ~50% of the magnocellular neurons recorded at resting membrane potential. The decrease in conductance and the inward current were caused largely by the attenuation of a resting potassium conductance because they were reduced by the replacement of intracellular potassium with an equimolar concentration of cesium or by the addition of potassium channel blockers to the extracellular medium.

In some cells, trans-ACPD still elicited a small inward current after blockade of potassium currents, which was abolished by the calcium channel blocker, CdCl2. Trans-ACPD also reduced voltage-gated and Ca2+-activated K+ currents in these cells. Trans-ACPD reduced the transient outward current (Ih, outward) by 10–70% and/or the Ito-mediated delay to spike generation in ~60% of magnocellular neurons tested. The cells that showed a reduction of Ih generally also showed a 20–60% reduction in a voltage-gated, sustained outward current. Finally, trans-ACPD attenuated the Ca2+-dependent outward current responsible for the afterhyperpolarization (IAHP) in ~60% of cells tested. This often revealed an underlying inward current thought to be responsible for the depolarizing afterpotential seen in some magnocellular neurons. (RS)-3,5-dihydroxyphenylglycine, a group I receptor-selective agonist, mimicked the effects of trans-ACPD on the resting and voltage-gated K+ currents. (RS)-α-methyl-4-carboxyphenylglycine, a group II metabotropic glutamate receptor antagonist, blocked these effects. A group II receptor agonist, 2S,1’S,2’S-2-carboxycyclopropylglycine and a group III receptor agonist, t. (+)-2-amino-4-phosphonobutric acid, had no effect on the resting or voltage-gated K+ currents, indicating that the reduction of K+ currents was mediated by group I receptors. About 80% of the SON cells that were labeled immunohistochemically for vasopressin responded to metabotropic glutamate receptor activation, whereas only 33% of labeled oxytocin cells responded, suggesting that metabotropic receptors are expressed preferentially in vasopressinergic neurons. These data indicate that activation of the group I metabotropic glutamate receptors leads to an increase in the postsynaptic excitability of magnocellular neurons by blocking resting K+ currents as well as by reducing voltage-gated and Ca2+-activated K+ currents.

INTRODUCTION

Hypothalamic neuroendocrine cells display bursting activities that are fashioned by a combination of intrinsic and synaptic mechanisms. The magnocellular neurons of the supraoptic nucleus (SON) and paraventricular nucleus are among the most extensively studied of mammalian neuroendocrine cells. Although many of the intrinsic mechanisms involved in burst generation in magnocellular neurons have been described, relatively little is known about the modulatory regulation of these cells by the diverse neurotransmitter inputs converging on them (Renaud and Bourque 1991).

Magnocellular neurons of the SON receive a dense glutamatergic innervation (Meeker et al. 1989), and more than one-third of the synapses in the SON are immunoreactive for glutamate (Meeker et al. 1993). Glutamate has been shown to be the main excitatory neurotransmitter in the hypothalamus through its actions at ionotropic glutamate receptors because blocking ionotropic receptors with selective antagonists completely abolishes spontaneous and evoked excitatory postsynaptic potentials and currents in hypothalamic neurons (Gribkoff and Dudek 1988, 1990; van den Pol et al. 1990; Wuarin and Dudek 1991, 1993). Ionotropic glutamate receptors also have been shown to play a major role in burst generation in vivo because N-methyl-D-aspartate and amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptor antagonists blocked bursting elicited in putative vasopressinergic magnocellular neurons by baroreceptor activation (Nis- sen et al. 1995).

Magnetocellular neurons of the SON express metabotropic glutamate receptors, albeit at a lesser density than that of the ionotropic glutamate receptors (Meeker et al. 1994). Anatomic and biochemical studies have provided evidence for four subtypes of metabotropic glutamate receptors in the hypothalamus: mGluR1 (Kiss et al. 1996; van den Pol 1994; van den Pol et al. 1994), mGluR3 (Tanabe et al. 1993), mGluR5 (Romano et al. 1995), and mGluR7 (Kinzie et al. 1995). Activation of metabotropic glutamate receptors in hypothalamic neurons has been shown to cause phosphoinositide hydrolysis (Sortino et al. 1991), to inhibit (cAMP) formation (Casabona et al. 1992), and to elicit an increase in intracellular unbound calcium (van den Pol et al. 1994). Very little, however, is known about the physiological effects of metabotropic receptor activation in the hypothalamus. Whereas ionotropic glutamate receptors are likely to play a large part in the initiation of bursts in magnocellular neurons, metabotropic glutamate receptors may be involved in shaping bursting patterns through slower modulatory actions.

Metabotropic glutamate receptors are coupled to intracel- lular signal transduction pathways through G proteins and are responsible for the modulatory actions of glutamate (Schoepf and Conn 1993). Eight subtypes of metabotropic...
glutamate receptor have been identified and classified into three groups on the basis of pharmacological properties and sequence homology. Group I metabotropic receptors include mGluR1 and mGluR5, which activate inositol trisphosphate production; group II receptors, composed of mGluR2 and mGluR3, and group III receptors, including mGluR4, -6, -7, and -8, are negatively coupled to cAMP (see Pin and Duvoisin 1995).

Many of the same intrinsic currents that are involved in bursting activity in magnocellular neurons (Renaud and Bourque 1991) are modulated by metabotropic receptor activation in neurons in other areas of the brain. Activation of metabotropic glutamate receptors reduces leak $K^+$ currents (Guérineau et al. 1994) and voltage-dependent and $Ca^{2+}$-activated $K^+$ currents ($I_{\text{AMP}}$) in hippocampal pyramidal cells, causing an increase in input resistance and a slow depolarization (Charpak et al. 1990; Constanti and Libri 1992; Desai and Conn 1991). Leak $K^+$ currents in neurons of the nucleus of the tractus solitarius (Glaum and Miller 1992) and dorsal-lateral LGN (McCormick and von Krosigk 1992), and peak and steady state voltage-gated $K^+$ currents in visceral sensory neurons in the nucleus of the solitary tract (Hay and Lindsey 1995) also are reduced by metabotropic receptor activation.

We studied the modulatory actions of metabotropic glutamate receptors on the postsynaptic currents of SON neurons with whole cell patch-clamp and conventional intracellular recording techniques in thick slices. We found that activation of group I metabotropic receptors in the hypothalamus increased the excitability of magnocellular neurons by reducing leak, voltage-gated, and $Ca^{2+}$-activated $K^+$ currents. Postsynaptic metabotropic glutamate receptors were expressed more prevalently in vasopressinergic neurons than in oxytocinergic neurons of the SON. Preliminary accounts of this study have been presented in abstract form (Schrader and Tasker 1994–1996).

**METHODS**

**Slice preparation**

Male Sprague Dawley rats (40–150 g) were anesthetized deeply with pentobarbital sodium (50 mg/kg body wt) and decapitated. The brain was removed rapidly and placed in cold (0–1°C), oxygenated artificial cerebrospinal fluid (ACSF), which contained (in mM) 124 NaCl, 3 KCl, 2.4 CaCl$_2$, 1.3 MgSO$_4$, 1.4 NaH$_2$PO$_4$, 11 glucose, and 5 $N$-[2-hydroxyethyl]piperazine-$N^\text{\textprime}$-[2-ethanesulfonic acid] (HEPES); pH was adjusted to 7.2–7.4 with NaOH.

A tissue block containing the hypothalamus was prepared by making razor cuts rostral and lateral to the optic chiasm, caudally at the level of the median eminence and dorsal to the third ventricle. The block was glued to the chuck of a vibrating tissue slicer (Campden Instruments) and rapidly submerged in ice-cold ACSF. Two coronal slices (400 $\mu$m) containing the SON were sectioned, (L-CCG-I, 1–100 M$m$) and 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20–50 M$m$) were bath applied to block ionotropic glutamate receptors in some experiments. Tetrodotoxin (TTX, 1–3 M$m$) was added to the bath in the voltage-clamp experiments to block voltage-gated Na$^+$ channels and spike-mediated synaptic transmission. TTX, L-CCG-I, and D-AP5 were dissolved in distilled water and frozen in 100-fold aliquots until the time of the experiment. DNQX was dissolved in dimethyl sulfoxide and frozen.

**Electrophysiology**

Sharp microelectrodes (120–180 M$m$) were pulled from thick-walled microfilament capillary tubes (1.0 mm OD, World Precision Instruments) on a Flaming-Brown puller (Sutter Instruments). The electrodes were filled with 2 M potassium acetate containing 1–2% biocytin. Microelectrodes were advanced through the tissue in 4-µm steps with a piezoelectric microdrive (Nanosteppe. Scientific Precision Instruments) set at maximum speed and acceleration settings. Conventional intracellular recordings with sharp microelectrodes were performed with an Axoclamp 2B amplifier (Axon Instruments).

Patch electrodes (4–8 M$m$) were made from borosilicate glass (1.65 mm OD, 1.2 mm ID, KG-33; Garner Glass) and also were pulled on a Flaming-Brown puller (Sutter Instruments). The pette solution contained (in mM) 120 potassium glutonate, 10 HEPES, 1 NaCl, 1 CaCl$_2$, 1 MgCl$_2$, 2 ATP (ATP, magnesium salt), 0.3 GTP (GTP, sodium salt), and 10 ethylene glycol bis-[β-(aminoethyl) ether]-N,N,N$^\text{\textprime}$,N$^\text{\textprime}$-tetraacetic acid (EGTA); pH was adjusted to 7.2–7.4 with KOH. In experiments in which Ca$^{2+}$-dependent K$^+$ currents were studied, EGTA was omitted from the electrode, and the potassium glutonate concentration was increased to 130 mM. Potassium currents were blocked in some experiments with a patch solution containing (in mM) 110 D-gluconic acid, 110 CsOH, 10 CsCl, 10 HEPES, 11 EGTA, 1 MgCl$_2$, 1 CaCl$_2$, 2 ATP, and 0.3 GTP; pH was adjusted to 7.2–7.4 with CsOH. Bio- cytin (0.1–0.3%) was included in patch pipettes as an intracellular marker.

Patch pipettes were advanced through the slice in 2-µm steps using a piezoelectric microdrive (Nanosteppe, Scientific Precision Instruments) set at minimum speed and acceleration settings. High-resistance seals (>1 G$m$) were obtained before going to the whole cell configuration. Series resistance ranged from 12 to 40 M$m$ and was compensated 60–80%. Whole cell data usually were recorded with an Axoclamp 2B amplifier in current-clamp experiments and with an Axopatch 1D amplifier (Axon Instruments) in voltage-clamp experiments.

All data were low-pass filtered at 2 kHz, digitized at 22 kHz, and stored on videotape. Selected data were digitized and analyzed using a DOS-based, 80486 personal computer with the Digidata 1200 interface and pCLAMP software (Axon Instruments). Current-voltage curves were generated on-line using the pCLAMP software to trigger voltage or current pulses. Input resistance was determined by measuring the voltage response to a −50-pA current pulse in current clamp, or the current generated by a −20- or −30-mV voltage step in voltage clamp. Liquid junction potentials (11 mV) were corrected according to Neher (1992).

Extracellular electrical stimulation was applied to the region dorsolateral to the SON using a bipolar electrode made from two tightly wound, teflon-coated platinum iridium wires (75 $\mu$m diam). Repetitive stimuli (100–500 $\mu$A, 0.5 ms) were delivered at a frequency of 10–20 Hz for 2–10 s.

**Pharmacology**

Trans-(-)-1-amino-1,3-cyclopentane dicarboxylic acid (trans-ACPD, 10–100 M$m$), (RS)-α-methyl-4-carboxypenylglycine (MCPG, 500–1000 M$m$), (RS)-3,5-dihydroxyphenylglycine (DHPG, 10–100 M$m$), 25S,1’S,2’S,2’s-2-carboxycyclopropylglycine (L-CCG-I, 1–100 M$m$), and L(+)-2-amino-4-phosphonobutyric acid (L-AP4, 100–250 M$m$) (Tocris Cookson or RBI) were applied to the perfusion bath; trans-ACPD was sometimes pressure applied focally in microdrops (100 M$m$) on the surface of slices. The ionotropic glutamate receptor antagonists d(-)-2-amino-5-phosphonopentanoic acid (d-AP5, 100 M$m$) and 6,7-dimethoxyquinoline-2,3-dione (DNQX, 20–50 M$m$) were bath applied to block ionotropic glutamate receptors in some experiments. Tetrodotoxin (TTX, 1–3 M$m$) was added to the bath in the voltage-clamp experiments to block voltage-gated Na$^+$ channels and spike-mediated synaptic transmission. TTX, L-CCG-I, and d-AP5 were dissolved in distilled water and frozen in 100-fold aliquots until the time of the experiment. DNQX was dissolved in dimethyl sulfoxide and frozen.
in 100× aliquots. In certain experiments, K⁺ channels were blocked with 20 mM tetraethylammonium chloride (TEA), substituted equimolar for NaCl in the ACSF, or with bath application of 20 mM TEA and 2 mM CsCl; 6 mM 4-aminopyridine (4-AP) also was added to the bath in some experiments to block the transient K⁺ current.

Intracellular labeling and immunohistochemistry

Cells were dialyzed with biocytin (0.1–0.2%) during most recordings (Horikawa and Armstrong 1988). After experiments, slices were removed from the recording chamber and fixed overnight in 4% paraformaldehyde dissolved in 0.1 M phosphate-buffered saline (PBS). They then were sectioned (15–20 μm) on a freezing microtome and the biocytin-injected cells were labeled by incubating the slices for 4 h in streptavidin-conjugated 7-amino-4-methyl-coumarin-3-acetic acid (AMCA, Molecular Probes). The AMCA was diluted (1:300) in 0.1 M PBS containing 1% Triton X. Sections were examined under a microscope equipped with epifluorescence using a 340–380 nm excitation/430 nm barrier filter combination to detect the presence of biocytin-filled, AMCA-labeled neurons.

Sections containing biocytin-filled cells then were submitted to one of two immunohistochemical double-labeling procedures using polyclonal and monoclonal antibodies, generously donated by Dr. H. Gainer of the National Institutes of Health. If the biocytin-filled cell was recovered in two serial sections, one section was treated with a rabbit polyclonal antibody for oxytocin (VA10) and the second section was treated with a rabbit polyclonal antibody for vasopressin (VA4) for 36 h at 4°C (both antibodies were diluted 1:1,000 in 0.1 M PBS with 1% normal sheep serum and 0.2% sodium azide). If the biocytin-labeled cell was recovered in a single section, that section was incubated in the rabbit polyclonal antibody to oxytocin (1:2,000) simultaneously with a monoclonal antibody to vasopressin-associated neurophysin (PS41; 1:200 in 0.1 M PBS with 1% normal sheep serum and 0.2% sodium azide) for 36 h at 4°C. The polyclonal antibodies have been tested for specificity with quantitative liquid phase radioimmunoassay by Gainer’s group (Alstein et al. 1988) and with preadsorption controls in our slices (Boudaba et al. 1996). The monoclonal antibody to vasopressin-neurophysin has been extensively tested for specificity in Gainer’s laboratory (Ben-Barak et al. 1985), and labels cells in the SON that are clearly distinct from cells labeled by the polyclonal oxytocin antibody in our slices (e.g., see Fig. 7). After PBS rinses, the polyclonal oxytocin and vasopressin antibodies were labeled with a goat anti-rabbit secondary antibody conjugated to fluorescein isothiocyanate (FITC; 1:100) and the monoclonal vasopressin-neurophysin antibody was labeled with a goat antimouse secondary antibody conjugated to rhodamine isothiocyanate (RITC; 1:200, 1 h). The sections were rinsed again in PBS, mounted onto slides, and coverslipped with Vectashield antifading mounting medium (Vector Labs). They then were examined under epifluorescence for the presence of biocytin labeling with the 340–380/430 nm filters (AMCA), and oxytocin or vasopressin immunoreactivity by switching to 450–490 nm excitation/515 nm barrier filters (FITC) or to 515–560 nm excitation/580 nm barrier filters (RITC).

Results

Recorded neurons were identified initially during experiments as SON magnocellular neurons by the presence of a transient, voltage-dependent K⁺ current, Iₖ (Bourque 1988; Cobbett et al. 1989; Tasker and Dudek 1991). A total of 78 putative magnocellular neurons were tested for their response to activation of metabotropic glutamate receptors in recordings that lasted from 30 to 100 min. Sixty-nine neurons were recorded with patch electrodes and had a mean membrane potential of −61.8 ± 1.1 mV (mean ± SE; n = 50) and a mean input resistance of 905 ± 51 MΩ (n = 59). Nine neurons were recorded with sharp electrodes to control for washout; these cells had a mean membrane potential of −54 ± 2.9 mV (n = 9) and a mean input resistance of 244 ± 34 MΩ (n = 9). No obvious differences in the current-clamp responses to metabotropic receptor activation were seen between cells recorded with sharp electrodes and those recorded with patch electrodes, providing evidence that washout of the metabotropic receptor signaling mechanisms did not occur. Furthermore, no washout of the metabotropic-receptor actions was seen in patch-clamped neurons tested up to 60 min into recordings.

Effects of activation of metabotropic glutamate receptors were first tested with the broad-spectrum agonist, trans-ACPD. Involvement in the observed responses of specific metabotropic receptor subtypes then was tested using subtype-selective agonists and antagonists. The Na⁺ channel blocker, TTX, had no significant effect on the response to trans-ACPD, and therefore was used to block Na⁺-dependent spikes and spike-mediated synaptic transmission in all voltage-clamp experiments. Current-clamp responses recorded with sharp electrodes and with patch electrodes were pooled for analysis.

Resting currents

Seventeen of 30 magnocellular neurons recorded in current clamp showed a 3–18 mV (6.9 ± 0.8 mV) depolarization accompanied by an increase in input resistance of 10–35% (18.5 ± 1.7%; Fig. 1A) in response to bath application of trans-ACPD. Eleven of 23 neurons recorded in voltage clamp showed a 5–40 pA (18.7 ± 3.45 pA) inward current and a 10–30% (16 ± 1.2%) decrease in input conductance in response to bath application of trans-ACPD (Fig. 1B). This inward current was larger at depolarized holding potentials, suggesting that it and the decrease in conductance were...
caused by the closing of K$^+$ channels. The inward current and depolarization were not due to activation of ionotropic glutamate receptors because the effect of trans-ACPD was not attenuated in the presence of the glutamate receptor antagonists, DNQX and d-AP5 ($n = 2$, data not shown). Repeated and prolonged applications (up to 3 successive applications lasting up to 15 min) of trans-ACPD revealed no apparent desensitization of the metabotropic glutamate receptors.

We tested for the K$^+$ dependence of the inward current and the decrease in conductance seen in response to trans-ACPD. Replacement of K$^+$ with Cs$^+$ in the recording electrode ($n = 10$) or bath application of TEA (20 mM) and CsCl (2 mM) ($n = 3$) or bath application of TEA (20 mM), CsCl (2 mM), and 4-AP (6 mM) ($n = 3$) reduced or blocked the inward current and the decreased conductance seen in trans-ACPD (Fig. 2) in all the cells tested. However, blocking K$^+$ channels failed to abolish completely the inward current caused by trans-ACPD in 4 of the 16 cells (25%) tested; in these cells, trans-ACPD caused a 5- to 20- pA inward current and a 5–24% increase in input conduc-

tance at resting potential (data not shown). This residual current was blocked by the calcium channel blocker, Cd$^{2+}$ (200 $\mu$M, $n = 2$), suggesting that it was caused by the activation or enhancement by trans-ACPD of a Ca$^{2+}$-dependent conductance.

**Transient K$^+$ current**

SON magnocellular neurons express a prominent transient K$^+$ current, $I_A$ (Bourque 1988; Cobbett et al. 1989). Activation of $I_A$ is characterized by a dampening of current-evoked depolarization and a delay in the onset of spike firing in current clamp and a rapidly inactivating outward current in voltage-clamp. Trans-ACPD reduced or blocked the delay-to-onset of spike firing in 12 of 21 neurons recorded in current clamp (Fig. 3A) and attenuated $I_A$ by 20–80% (57.3 ± 5.9% ) in 11 of 21 cells recorded in voltage clamp (Fig. 3B). The $I_A$ was evoked by 30- to 50-mV depolarizing steps from a holding potential of −80 to −90 mV; it was blocked by 4-AP (6 mM, $n = 3$, data not shown).

**Sustained K$^+$ current**

A sustained outward current also was generated when the cells were given depolarizing voltage steps from a holding potential of −80 to −90 mV. The sustained outward current was blocked by TEA (20 mM) and CsCl (2 mM, $n = 6$, data not shown), suggesting that it was a K$^+$ current. Blockade of the sustained K$^+$ current with TEA and CsCl revealed a high-voltage–activated Ca$^{2+}$ current, which was not under voltage control and was not studied in these experiments. The sustained K$^+$ current was similar to the delayed rectifier described in magnocellular neurons by Cobbett et al. (1989), although it was a nonactivating current in our experiments, suggesting that more than one type of outwardly rectifying K$^+$ conductance may contribute to the current. A sustained K$^+$ current has been hypothesized to mediate an outward rectification that is specific to oxytocinergic neurons of the SON (Stern and Armstrong 1997), although we found the sustained current to be expressed in SON vasopressinergic neurons as well (see below). Trans-ACPD reduced this sustained K$^+$ current by 20–75% (52 ± 5.8%) in 8 of 16 magnocellular neurons tested (Fig. 3C). The reduction in the sustained K$^+$ current by trans-ACPD usually, but not always, accompanied the reduction in $I_A$.

**Ca$^{2+}$-dependent K$^+$ current**

We also studied the Ca$^{2+}$-dependent K$^+$ current responsible for the afterhyperpolarization ($I_{AHP}$) (Andrew and Dudek 1984; Bourque 1988; Cobbett et al. 1989) in recordings in which the Ca$^{2+}$ chelator, EGTA, was omitted from the patch solution. Afterhyperpolarizations were evoked in current clamp by eliciting trains of 6–10 action potentials with depolarizing current pulses (150–300 ms). In voltage-clamp experiments, the $I_{AHP}$ was generated with a 40- to 60-mV depolarizing voltage step (150–500 ms) from a holding potential of −30 to −40 mV. The $I_{AHP}$ was blocked by Cd$^{2+}$ (200 $\mu$M) or by total replacement of extracellular K$^+$ and Na$^+$ with TEA (120 mM) and N-methyl-D-glucamine (10 mM) ($n = 3$). The AHP was reduced by trans-ACPD in two of five cells tested in current clamp (Fig. 4A). In voltage-clamp recordings, trans-ACPD reduced the $I_{AHP}$ by >60% in four
of six neurons; in two of these six neurons, the I_{AHP} was blocked completely, revealing an underlying inward current (Fig. 4B). This inward current is presumably responsible for the depolarizing afterpotential observed in some magnocellular neurons (Andrew and Dudek 1984; Bourque 1986).

**Group I receptors mediate metabotropic receptor actions on K⁺ currents**

We investigated the receptor subtypes responsible for the effects of activation of metabotropic glutamate receptors by trans-ACPD. DHPG, an agonist specific for group I receptors (Ito et al. 1992), mimicked the effects of trans-ACPD on leak currents as well as on voltage-gated and Ca²⁺-activated K⁺ currents. DHPG (50–100 μM) caused an 8-to-36-pA (16.17 ± 4.25 pA) inward current and a 10–35% (30 ± 2%) decrease in conductance in 6 of 16 cells. DHPG also reduced the I₄ and the sustained K⁺ current in 9 of 14 cells by 30–70% (I₄; 42 ± 4.8%; sustained K⁺ current: 51 ± 5%) (Fig. 5) and reduced the Ca²⁺-activated K⁺ current by >30% in 2 of 4 cells. The group I/II metabotropic receptor antagonist, MCPG (500 μM) (Eaton et al. 1993b), blocked the reduction in K⁺ currents caused by DHPG (Fig. 6; n = 3). L-CCG-I, a group II receptor agonist (Hayashi et al. 1992), had no consistent effect at any of the concentrations tested (1 μM: n = 2; 10 μM: n = 6; 100 μM: n = 2). L-AP4 (100–250 μM), a group III receptor-selective agonist (Thomsen et al. 1992), had a significant presynaptic effect (Schrader and Tasker 1997), but it too had no apparent effect on postsynaptic currents in SON magnocellular neurons (n = 5). Thus the reduction in K⁺ currents caused by metabotropic receptor activation appeared to be mediated by the group I receptors, mGluR1 and/or mGluR5.

**Activation of metabotropic glutamate receptors with endogenous glutamate**

The mGluR1 splice variant, mGluR1α, is located at the periphery of the synaptic junction in other areas of the brain (Baude et al. 1993), suggesting that these receptors may not be activated with low levels of glutamate release, such as those expected during low-frequency spiking in presynaptic neurons. Rather, higher concentrations of glutamate in the synaptic cleft resulting from repetitive spiking activity in the presynaptic neurons might be required to allow released glutamate to diffuse to extrasynaptic metabotropic glutamate receptors. Because repetitive, high-frequency stimulation of glutamatergic inputs to the SON evokes a slow depolarization in magnocellular neurons (Dudek and Gribkoff 1987), we hypothesized that this response might be caused by activation of extrasynaptic metabotropic glutamate receptors. To test this hypothesis, we applied repetitive extracellular stimuli (10–20 Hz, 2–10 s) dorsolateral to the SON, which elicited a slow inward current and a reduction of I₄. The metabotropic glutamate receptor antagonist MCPG (500–1,000 μM) had no effect on either the slow inward current or on the I₄ (n = 6; data not shown), indicating that they were not caused by activation of the metabotropic glutamate

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**Fig. 3.** Metabotropic receptor activation reduced the transient and sustained voltage-gated K⁺ currents. A: depolarization of a magnocellular neuron with a 100-pA current pulse from a membrane potential of −75 mV generated a train of action potentials following a delay (●) caused by activation of the transient K⁺ current (Control). Bath application of 100 μM trans-ACPD blocked the delay (Trans-ACPD), which reappeared (○) with washout of the trans-ACPD (Wash). Membrane potential was maintained at −75 mV throughout the experiment with negative current injection. B: another magnocellular neuron was voltage clamped at −90 mV and stepped to −40 mV to evoke a transient outward current (●) in normal ACSF (Control). Bath application of trans-ACPD (100 μM) reduced the transient outward current (Trans-ACPD), and the effect reversed with washout (Wash). Each trace is an average of 5 responses. C: depolarizing steps (30–70 mV by 10-mV increments, 2.5-s duration), from a conditioning potential of −80 mV (200 ms), elicited a noninactivating outward current (●) in normal ACSF (Control). Bath application of trans-ACPD (100 μM) reduced the sustained outward current (Trans-ACPD), which returned with washout of the trans-ACPD (Wash). Each trace is an average of 5 responses. D: population current-voltage plot showing, across cells (n = 11), the mean peak amplitude (±SE) of the transient K⁺ current evoked at different test potentials in control ACSF (●) and in trans-ACPD (○). Amplitude values represent the peak of the total current at each test potential. E: population current-voltage plot (n = 6 cells) showing the mean amplitude (±SE) of the sustained K⁺ current evoked at different test potentials in control ACSF (●) and in trans-ACPD (○). Amplitude values are the average current during the last 50 ms of the response.
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FIG. 4. Metabotropic receptor activation blocked the Ca²⁺-activated K⁺ current. A: a 100-pA, 250-ms current pulse delivered in current clamp caused a train of 7 action potentials followed by an afterhyperpolarization (Control). Cell was held at a depolarized membrane potential (~45 mV). Trans-ACPD (100 μM) application attenuated the afterhyperpolarization (Trans-ACPD) generated by the same number of action potentials at the same membrane potential (~45 mV). Action potentials are truncated. B: a 55-mV, 150-ms depolarizing step (not shown), delivered from a holding potential of ~45 mV, elicited a small outward tail current, the Ca²⁺-activated K⁺ current (I_AHP), after repolarization to ~45 mV (Control). Bath application of Trans-ACPD (100 μM) blocked the I_AHP and revealed an inward tail current (Trans-ACPD), which corresponded presumably to the current responsible for the depolarizing afterpotential. Blockade of the I_AHP reversed with washout of the trans-ACPD (Wash). Traces were superimposed manually to compensate for the change in holding current caused by trans-ACPD.

receptors. In four of the six experiments, the ionotropic glutamate receptor antagonists DNQX (50 μM) and AP5 (100 μM) were included in the bathing medium to block fast excitatory synaptic responses. In the other two experiments, the γ-aminobutyric acid-A–receptor antagonist bicuculline (30 μM) and the ionotropic antagonists were included to block both fast excitatory and fast inhibitory responses. No evidence for the activation of the postsynaptic metabotropic receptors by endogenously released glutamate was found in any of the experiments.

Identification of magnocellular neurons

Phasic burst generation generally is considered to be characteristic of vasopressinergic magnocellular neurons (Cobett et al. 1986; Poullain and Wakerley 1982; Yamashita et al. 1983). Of five putative vasopressin neurons that could be induced to fire phasically with intracellular current injec-

FIG. 5. Postsynaptic effects of metabotropic receptor activation are mediated by group I receptors. Group I receptor agonist, (RS)-3,5-dihydroxypheynylglutamic acid (DHPG), mimicked the effects of trans-ACPD on K⁺ currents. A: application of DHPG (50 μM) reversibly reduced the transient K⁺ current (→) elicited with a 30-mV depolarizing step from a holding potential of ~80 mV. Each trace is the average of 5 responses. B: DHPG application (50 μM) also reduced the sustained K⁺ current (→) evoked in the same cell with 30- to 70-mV depolarizing steps (in 10-mV increments) from a holding potential of ~80 mV. Each trace is the average of 5 responses. C: population current-voltage plot showing, across cells (n = 7), the mean current amplitude (±SE) at the peak of the transient K⁺ current elicited at different test potentials in normal ACSF (□) and in DHPG (●). Currents were elicited with depolarizing steps from a 200-ms conditioning step to ~80 to ~90 mV and were measured at the peak of the total current. D: population current-voltage plot (n = 9 cells) showing the mean amplitude of the sustained K⁺ currents (±SE) evoked at different test potentials in normal ACSF (□) and during bath application of DHPG (●).

FIG. 6. Postsynaptic effects of group I receptor activation are blocked by the group I/II metabotropic glutamate receptor antagonist, (RS)-α-methyl-4-carboxyphenylglycine (MCPG). A: transient K⁺ current (→) elicited with a 35-mV depolarizing step from a holding potential of ~80 mV (Control) was reduced by DHPG application (50 μM; DHPG). It recovered with washout (45 min) of the DHPG (Wash); MCPG (500 μM) had no effect on the transient outward current (MCPG) but blocked the effect of DHPG (MCPG + DHPG). B: application of DHPG also reduced the sustained K⁺ current (→) elicited with a series of 10- to 60-mV depolarizing steps, in 10-mV increments, from a holding potential of ~70 mV in the same cell, and this also was blocked by MCPG. Each trace in A and B is the average of 5 responses.
FIG. 7. Immunohistochemical identification of a vasopressinergic neuron that responded to metabotropic glutamate receptor activation. A magnocellular neuron that showed a postsynaptic response to trans-ACPD was injected with biocytin, labeled, and subjected to immunohistochemical double-labeling with antibodies to oxytocin and vasopressin. A1–C1: at low magnification to show the SON; A2–C2: at higher magnification. A, 1 and 2: biocytin-filled cell was labeled with the blue fluorescent marker, 7-amino-4-methyl-coumarin-3-acetic acid (AMCA), and visualized under UV filters. The biocytin-labeled cell was distinguished readily by its color and intensity of fluorescence from the FITC-labeled cells that bled through the UV filter combination. B, 1 and 2: same section viewed under fluorescein filters to visualize the fluorescein isothiocyanate (FITC)-labeled, oxytocin-positive cells. The biocytin-labeled cell was not double labeled with FITC (r), indicating that it was oxytocin negative. C, 1 and 2: same section viewed under rhodamine filters showed the RITC-labeled, vasopressin-positive neurons. The biocytin-labeled cell was double labeled with rhodamine isothiocyanate (r), indicating that it was vasopressin positive. Calibration in A1 applies to A1–C1 and that in A2 applies to A2–C2.

Immunohistochemical studies also have shown mRNA for receptor subtype mGluR1a to be expressed, albeit at varying concentrations, in the SON (Kiss et al. 1996; van den Pol 1994). Our results, in light of these findings, suggest that mGluR1 receptors may be coupled through signal transduction pathways to K⁺ channels in SON magnocellular neurons. The lack of effect of the group II receptor agonist, L-CCG-I, is consistent with the lack of expression of mGluR2 mRNA (Ohishi et al. 1993), but not with the presence of mGluR3 mRNA in the SON (Serje et al. 1996; Tanabe et al. 1993). One possible explanation for this discrepancy is that mGluR3 receptors are localized to glial cells rather than to neurons, as suggested by Tanabe et al. (1993). Similarly, the group III receptor agonist, L-AP4, had no discernible effect on the intrinsic membrane currents of SON neurons, whereas mGluR7 mRNA expression has been described in the SON (Kinzie et al. 1995). We found in a recent study that activation of group III metabotropic receptors at presynaptic terminals in the SON caused a reduction in transmitter release and that this probably was mediated by mGluR7 (Schrader and Tasker 1997). However, this would not account for mGluR7 mRNA in the SON because the mRNA presumably would be constrained to the presynaptic cell bodies. The expression of mGluR7 mRNA in the SON and the lack of physiological evidence for mGluR7 receptors on the magnocellular neurons in our experiments suggest that the receptors may be localized to the axon terminals in the neurohypophysis, which were not present in our slices.

Metabotropic glutamate receptor subtypes

We found that the reduction in K⁺ currents by metabotropic receptor activation was mediated by group I receptor subtypes (i.e., mGluR1 and/or mGluR5), whereas activation of group II and group III receptors had no direct effects on SON magnocellular neurons. In situ hybridization histochemistry has revealed mRNA for receptor subtype mGluR1 in the SON (Serje et al. 1996), whereas mGluR5 protein expression appears to be minimal (Romano et al. 1995). A total of 18 SON cells were identified immunohistochemically as vasopressinergic (n = 9) or oxytocinergic (n = 9). About 80% of the vasopressin-positive cells (7 of 9) responded to metabotropic glutamate receptor activation (Fig. 7). Only 33% of the oxytocin-positive cells (3 of 9), on the other hand, were responsive. These data, together, suggest that metabotropic glutamate receptors are expressed preferentially, although perhaps not exclusively, in vasopressinergic magnocellular neurons.

DISCUSSION

The data presented here show that activation of metabotropic glutamate receptors reduces leak, voltage-gated and Ca²⁺-activated K⁺ conductances in SON magnocellular neurons. These effects persisted in the presence of TTX and could be distinguished from the actions of metabotropic glutamate receptors located at presynaptic terminals or at presynaptic somata/dendrites (Schrader and Tasker 1997), indicating that they were mediated by postsynaptic metabotropic glutamate receptors.

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We found that the reduction in K⁺ currents by metabotropic receptor activation was mediated by group I receptor subtypes (i.e., mGluR1 and/or mGluR5), whereas activation of group II and group III receptors had no direct effects on SON magnocellular neurons. In situ hybridization histochemistry has revealed mRNA for receptor subtype mGluR1 in the SON (Serje et al. 1996), whereas mGluR5 protein expression appears to be minimal (Romano et al. 1995). Immunohistochemical studies also have shown mGluR1α to be expressed, albeit at varying concentrations, in the SON (Kiss et al. 1996; van den Pol 1994). Our results, in light of these findings, suggest that mGluR1 receptors may be coupled through signal transduction pathways to K⁺ channels in SON magnocellular neurons. The lack of effect of the group II receptor agonist, L-CCG-I, is consistent with the lack of expression of mGluR2 mRNA (Ohishi et al. 1993), but not with the presence of mGluR3 mRNA in the SON (Serje et al. 1996; Tanabe et al. 1993). One possible explanation for this discrepancy is that mGluR3 receptors are localized to glial cells rather than to neurons, as suggested by Tanabe et al. (1993). Similarly, the group III receptor agonist, L-AP4, had no discernible effect on the intrinsic membrane currents of SON neurons, whereas mGluR7 mRNA expression has been described in the SON (Kinzie et al. 1995). We found in a recent study that activation of group III metabotropic receptors at presynaptic terminals in the SON caused a reduction in transmitter release and that this probably was mediated by mGluR7 (Schrader and Tasker 1997). However, this would not account for mGluR7 mRNA in the SON because the mRNA presumably would be constrained to the presynaptic cell bodies. The expression of mGluR7 mRNA in the SON and the lack of physiological evidence for mGluR7 receptors on the magnocellular neurons in our experiments suggest that the receptors may be localized to the axon terminals in the neurohypophysis, which were not present in our slices.
Metabotropic receptor actions in oxytocin and vasopressin neurons

That metabotropic receptor activation reduced K⁺ currents in approximately half of the SON cells tested led us to hypothesize that the effect was specific to either oxytocin or vasopressin neurons because there are approximately equal numbers of oxytocin and vasopressin neurons in the SON (Vandesande and Diericks 1975). Immunohistochemical staining after biocytin injections indicated that 78% of the cells that were vasopressin positive showed a postsynaptic response to metabotropic receptor activation, whereas only 33% of the oxytocin-positive cells were sensitive to metabotropic receptor activation. In addition, 80% of phasically firing cells (i.e., putative vasopressin cells) responded to metabotropic receptor activation. These results together suggest that the group I metabotropic receptors are expressed more prevalently in vasopressin than in oxytocin neurons and are consistent with the lack of effect of metabotropic receptor activation in oxytocin neurons in organotypic slice cultures (Jourdain et al. 1996).

Activation of metabotropic glutamate receptors by endogenous glutamate

Postsynaptic metabotropic receptors have been localized to perisynaptic sites on the postsynaptic membrane (Baude et al. 1993), suggesting that they may not be activated by glutamate released during single presynaptic impulses. Like the presynaptic metabotropic receptors on glutamatergic terminals in the hippocampus (Scanziani et al. 1997), the postsynaptic receptors may require repetitive activation of presynaptic glutamate afferents and increased glutamate release and diffusion to be activated. Repetitive stimulation of synaptic inputs to hippocampal pyramidal neurons (Charpak and Gähwiler 1991) and to cerebellar Purkinje cells (Batchelor and Garthwaite 1993) causes a slow depolarization that mimics the depolarizing effect of exogenous activation of metabotropic glutamate receptors and that is blocked by MCPG (Batchelor et al. 1994). Similarly, the slow excitation elicited both with noxious stimuli and with exogenous activation of metabotropic receptors in neurons of the thalamus is blocked by the metabotropic receptor antagonists, (S)–4C3HPG and (S)–4CPG (Eaton et al. 1993a). Dudek and Gribkoff (1987) reported a similar synchronically activated, slow depolarization in magnocellular neurons of the SON in response to high-frequency extracellular stimulation. We studied this response in SON neurons for metabotropic receptor involvement by testing its sensitivity to blockade by MCPG because MCPG was effective in blocking the activation of postsynaptic metabotropic receptors by exogenous agonists. MCPG had no detectable effect on the fast excitatory synaptic currents elicited by single stimuli or on the slow inward current and reduction of Iᵥ generated by repetitive extracellular stimulation. The postsynaptic group I metabotropic receptors revealed in our experiments with exogenous agonists were therefore not activated by stimulus-evoked glutamate release. This suggests either that the metabotropic receptors on SON magnocellular neurons are located at a distance from the glutamate release site that precludes their activation by endogenously released glutamate, at least with the stimulation protocols we applied, or that they are present at synapses that were not activated by our stimulation.

Physiological significance

Changes in blood pressure or blood osmolality trigger the release of vasopressin from magnocellular neuroendocrine cells. This release of vasopressin is correlated with the adaptation of a phasic firing pattern by the vasopressin-releasing neurons (Poulin and Wakely 1982). Several lines of evidence indicate that metabotropic glutamate receptors may play a role in shaping the activity of vasopressinergic neurons generated in response to physiological demands, particularly during osmoregulation. First, the density of the metabotropic glutamate receptors in the hypothalamus is increased in response to water deprivation (Meeker et al. 1994b). Second, Serje et al. (1996) reported recently that metabotropic receptor activation causes an increase in vasopressin release from primary cultures of hypothalamic neuroendocrine cells. Our results suggest that metabotropic receptor activation may modulate the activity of vasopressinergic neurons, and thus influence vasopressin release, by increasing cell excitability and reducing voltage-gated and Ca²⁺-activated K⁺ conductances that shape bursting activity.

The modulatory role played by postsynaptic metabotropic receptors in the SON may be more important under conditions of osmoregulation because metabotropic receptors are upregulated by dehydration (Meeker et al. 1994b). We found no evidence for activation of metabotropic receptors by endogenously released glutamate, but all of our experiments were conducted in slices from normally hydrated rats. Future experiments using slices from dehydrated rats may reveal a physiological role of metabotropic glutamate receptors in the osmoregulatory response of vasopressin neurons.

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