Metabotropic Glutamate Receptors in Median Preoptic Neurons Modulate Neuronal Excitability and Glutamatergic and GABAergic Inputs From the Subfornical Organ

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INTRODUCTION

The neural tissue forming the lamina terminalis at the anterior wall of the third cerebral ventricle is recognized as a major contributor to homeostasis in several critical domains, notably hydromineral balance, neuroendocrine and cardiovascular functions, core temperature, and sleep-wake regulation. These functions involve the collective activities of neurons residing at three defined sites along the lamina terminalis. Two of these, the subfornical organ (SFO) located dorsally and the organum vasculosum lamina terminalis (OVLT) situated ventrally, are known as sensory circumventricular organs or CVOs where fenestrated capillaries and the lack of a blood-brain barrier permit neurons to sense via specific receptors the osmolality of the plasma and to detect circulating ligands for receptors on these neurons (reviewed in McKinley et al. 2003).

At the midpoint of the lamina terminalis and surrounding the anterior commissure is the median preoptic nucleus (MnPO, also called nucleus medianus, MnPN or POMe) containing neurons that receive inputs and interconnect with those in the SFO and OVLT as noted in both anatomical (e.g., Lind and Johnson 1982; Lind et al. 1982; Miselis et al. 1979; Oldfield et al. 1992) and functional studies (e.g., Gutman et al. 1989; Kolaj and Renaud 2007; Kolaj et al. 2004; Tanaka et al. 1987). MnPO neurons are suitably positioned to acquire information that derives from both hemal and neural origins. Additional afferent and efferent connectivity with neurons in hypothalamic and brain stem centers provide possible circuits whereby MnPO neurons can influence several aspects of homeostasis such as hydromineral and cardiovascular regulation, salt appetite, and sleep-waking behavior (Johnson et al. 1996; McKinley et al. 1996; Suntsova et al. 2002). Indeed major disruptions to homeostasis can be noted as a consequence of structural or chemical lesions focused on MnPO and the neighboring periventricular tissues (e.g., Bealer 2000; Cunningham et al. 1991; Gardiner et al. 1985, 1986; Mangiapane et al. 1983; Yasuda et al. 2000).

Knowledge of the neural mechanisms operating within the lamina terminalis has the potential to reveal not only how neurons along the lamina terminalis might sustain homeostasis but also provide insight into the pathophysiology of disorders such as essential and salt-sensitive hypertension (e.g., see Orlov and Mongin 2007). Initial interest in the neural connection between the SFO and the MnPO attracted attention after investigators noted that the integrity of this pathway was essential for drinking behavior triggered by systemic angiotensin (Eng and Miselis 1981; Lind and Johnson 1982; Mangiapane and Simpson 1980). Interestingly, recent investigations have revealed that neurons in the sensory CVOs, and the SFO in particular, are responsive to a variety of peptide hormones, implying roles in cardiovascular and body fluid homeostasis, feeding, and metabolism (for reviews, see Hoyda et al. 2009; McKinley and Oldfield 1998). Whereas the properties and neuropharmacology of SFO neurons have been revealed in some detail (see Washburn and Ferguson 2001), relatively little is known as to how information from SFO neurons is transmitted to target neurons along the lamina terminalis. MnPO is a principal target of SFO neuronal efferent fibers (Miselis et al. 1979). However, information on the nature of this connection at the cellular level is relatively scarce. In a recent in vitro analysis, we reported that electrical stimulation within SFO and along its ventral edge could evoked both GABAergic and glutamate ionotropic receptor-mediated rapid neurotransmis-
sion in MnPO neurons and noted that these evoked responses could be modulated by activation of metabotropic GABA<sub>B</sub> receptors (Kolaj et al. 2004). The preoptic area also displays immunoreactivity and mRNA for metabotropic glutamate receptors, or mGluRs (group I: van Den Pol 1994; van Den Pol et al. 1995; group II: Gu et al. 2008; group III: Ohishi et al. 1995). The observation that injections of (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD), a nonselective mGluR agonist, into MnPO produced a dose-dependent increase in plasma vasopressin, heart rate, and arterial pressure (Yamaguchi and Watanabe 2004), implies that these mGluRs do indeed have functional correlates. To gain a better understanding at the cellular level of the characteristics and potential roles of each group of mGluRs influencing the neural circuitry along the upper lamina terminalis, the present analysis evaluated the influence of exogenous application of selective mGluR agonists and antagonists on neuronal excitability in MnPO neurons and on their SFO-evoked glutamate- and GABA<sub>A</sub> receptor-mediated rapid postsynaptic events.

**METHODS**

Experimental protocols conforming to the Canadian Council for Animal Care guidelines were approved by the Ottawa Health Research Institute Animal Care and Use Committee. Care was taken to minimize the number of animals used and their suffering. Recordings were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004). Experiments used Wistar rats weighing 50–120 g (21–35 days old) and housed in pairs in a temperature-controlled environment under 12-h light/dark conditions. Brain slice preparations were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (Kolaj et al. 2004).

**RESULTS**

Data were obtained from MnPO neurons located dorsal (n = 8) or ventral (n = 72) to the anterior commissure. Because cells in both locations displayed similar properties, the data from all cells were pooled. Data samples obtained at the outset of whole cell recordings revealed a mean resting membrane potential of −55.3 ± 0.8 mV (n = 80) and input conductance of 1.25 ± 0.08 nS. Because preliminary observations with the cell-attached configuration revealed that bath applications of a nonselective mGluR agonist (1S,3R)-ACPD (50 μM) could either increase or decrease neuronal firing frequency, we were prompted to assess how cells responded to selective activation of different groups of mGluRs.

**Postsynaptic group I mGluR activation increases neuronal excitability**

Experiments in the cell-attached mode revealed that five MnPO neurons tested with bath applications of a selective group I mGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG; 5–10 μM for 2 min) responded with either the initiation of firing or a significant enhancement in ongoing action potential discharges that persisted for several minutes beyond wash (Fig. 1A). When tested under whole cell conditions in control ACSF, the response in 22/36 MnPO neurons to similar DHPG applications was a slowly developing, prolonged, and eventually reversible inward current, achieving a peak amplitude of −13.7 ± 1.8 pA (Fig. 1B). Similar inward currents obtained with DHPG applications in ACSF containing TTX (n = 9; P = 0.173) suggested that response to DHPG was direct and mediated by postsynaptic group I mGluR receptors. The DHPG-induced inward currents were significantly reduced when tested in the presence of each of two group I mGluR antagonists: (RS)-1-aminoindan-1,5-dicarbonyl acid (AIDA; 500 μM), a selective mGluR group I antagonist reduced the control response of −12.5 ± 2.6 to −4 ± 3.2 pA (P < 0.05; n = 4; Fig. 1C); 7-(hydroxyimino)cyclopropane [b]chroman-1a-carboxylate ethyl ester (CPCCOEt; 100 μM), a noncompetitive antagonist (preferably mGluR1 and not mGluR5), reduced the control response of −15.7 ± 4.7 to...
-7.7 ± 3.7 pA (P < 0.05; n = 3; Fig. 1C; P > 0.05 when comparing effects by AIDA and CPCCOEt). Analyses of the membrane conductances associated with DHPG-induced inward currents revealed three patterns. In 11/22 neurons, conductance decreased from 1.45 ± 0.18 to 1.12 ± 0.12 nS (P < 0.01) and I-V plots reversed approximately -100 ± 4 mV (n = 4; P < 0.05; Fig. 1E). In 7/22 neurons, conductance increased from 1.39 ± 0.23 to 1.63 ± 0.30 nS (P < 0.05) and I-V plots reversed at -33 ± 3 mV (n = 3). In the remaining four neurons, conductance changes (from 1.54 ± 0.32 to 1.56 ± 0.31 nS) were not significant, and I-V plots (n = 2) showed a parallel shift without reversal. Resting conductances and amplitudes of DHPG-induced inward currents were not significantly different between these three groups (1-way-ANOVA; conductance: F = 0.0798, P = 0.924; inward current: F = 0.785; P = 0.47). Collectively, the data suggest that group I mGluRs are postsynaptic and couple with at least two types of conductances, one that decreases and reverses close to the potassium equilibrium potential and another that increases and may be mediated by a nonelective cationic conductance.

Group I mGluR activation decreases SFO-evoked EPSCs

In previous investigations, we noted that electrical stimulation in the SFO or along its ventral border could elicit glutamate-mediated EPSCs or GABA$_A$ receptor-mediated IPSCs in MnPO neurons (Kolaj and Renaud 2007; Kolaj et al. 2004, 2007). Similar protocols were used here to evaluate the hypothesis that mGluRs might attenuate SFO-evoked inputs. Indeed as illustrated in Fig. 2, the addition of DHPG was associated with a gradual reduction in the amplitude of SFO-evoked EPSCs (70.5 ± 5.4% control; P < 0.01), slowly reversing on wash. This response was selective with no obvious influence on SFO-evoked IPSCs (Fig. 2, A–C). As mentioned in the preceding text, DHPG also induced inward currents in MnPO neurons, but we found no significant correlation between DHPG-induced inward currents and the decrease in SFO-evoked EPSCs (R = 0.423; P = 0.345; n = 7).

In the presence of the specific group I antagonist AIDA, a 70.1 ± 7.3% DHPG-induced reduction in SFO-evoked EPSCs was almost completely abolished (97.8 ± 2.3%; P < 0.05; n = 4). DHPG was also noted to influence spontaneous postsynaptic events. Two MnPO neurons displayed a decrease in spontaneous EPSC frequency (80 ± 4% of control) but not amplitude (98 ± 3% of control). Additionally, responses to DHPG in a subpopulation of MnPO neurons involved a significant increase in both frequency and amplitude of both spontaneous EPSCs (sEPSCs) and spontaneous IPSCs (sIPSCs). For sEPSCs,
frequency increased to $437 \pm 131\%$ of control ($P < 0.05$) and amplitude to $126 \pm 12\%$ of control ($P < 0.05; n = 3$). For sIPSCs, frequency increased to $145 \pm 12\%$ of control ($P < 0.05$) and amplitude to $111 \pm 2\%$ of control ($P < 0.05; n = 4$). These latter observations suggest that DHPG was acting at group I mGluRs to excite neurons that were afferent to the recorded MnPO neurons in our slice preparations.

Postsynaptic group II mGluR activation decreases neuronal excitability

When DCG IV (10 $\mu$M), a specific group II mGluR agonist was bath applied during cell attached recordings, all three spontaneously firing neurons tested displayed a reduction or transient cessation in their activity (Fig. 3A). Under whole cell recording conditions, 27/49 cells responded to bath applied DCG IV with a slowly rising and reversible outward current (mean: $7.3 \pm 0.6$ pA) accompanied by a 120.2 $\pm 3.2\%$ increase in membrane conductance ($P < 0.001$; Fig. 3, B–D). Similar trials in the presence of TTX revealed no significant change in DCG IV-induced outward current ($P = 0.158$) or associated membrane conductance ($P = 0.565; n = 5$), consistent with a postsynaptic location for group II mGluRs. Amplitudes of the outward current and membrane conductance change were correlated positively (Pearson correlation; $R = 0.426; P < 0.05; n = 27$). When re-tested in the presence of a highly selective group II mGluR antagonist (2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xan-th-9-yi) propanoic acid (LY 341495), both the control DCG IV-induced outward current ($7.4 \pm 1.9$ pA) and increase in membrane conductance ($117.7 \pm 1.9\%$) were significantly attenuated, to $1.9 \pm 0.4$ pA and $103.4 \pm 0.4\%$, respectively (Fig. 3C; $P < 0.001; n = 7$).

$I$-$V$ plots revealed a net DCG-induced current that reversed at $-96 \pm 2.4$ mV (cf. Fig. 3E; $n = 5$), approximating the potassium equilibrium potential ($E_K^-$) of $-98$ mV under these experimental conditions. These data suggest that activation of postsynaptic group II mGluRs in MnPO neurons promotes membrane hyperpolarization through opening of potassium channels.

We tested whether individual MnPO neurons might bear both group I and II mGluRs. When DHPG and DCG IV were applied in random order to 21 MnPO neurons, of the 18 cells that responded to at least one agonist, 6 responded to both, 7 responded to only DHPG, and the remaining 5 responded to DCG IV. These data suggest that the majority of MnPO neurons bear either group I or group II mGluRs with few expressing both.

**Group II mGluR activation decreases both SFO-evoked EPSCs and IPSCs**

Both SFO-evoked EPSCs and IPSCs displayed significant reductions in their amplitudes following addition of DCG IV (Fig. 4A). Responses developed slowly and were at least partially reversible after a 20-min wash. Reductions were significantly more prominent for EPSCs (to 48 $\pm 4.7\%$ of control) than for IPSCs (to 68.4 $\pm 4.4\%$ of control; $P < 0.01$; Fig. 4, B and C). Although a DCG IV-induced outward current was concurrent with the depression in SFO-evoked EPSCs (7/11 cells) or SFO-evoked IPSCs (9/14 cells), there was no significant correlation between the decrease in SFO-evoked postsynaptic currents and the increase in membrane conductance for either EPSCs ($r = -0.131; P = 0.718; n = 11$) or IPSCs ($r = 0.051; P = 0.877; n = 14$). The DCG IV-induced reductions in amplitude were reduced in the presence of the antagonist LY 341495 (EPSCs to 93.7 $\pm 3\%$; $P < 0.01; n = 4$; IPSCs to 96.3 $\pm 1.8\%$; $P < 0.05; n = 3$).

DCG IV also influenced spontaneous postsynaptic currents. For sEPSCs, frequency decreased to $61.6 \pm 7.3\%$ of control ($P < 0.05; n = 4$) and amplitude either decreased to $89 \pm 2\%$ of control ($P < 0.05; n = 2$) or showed no change (94 $\pm 3\%$ control; $P > 0.05; n = 2$). For sIPSCs, frequency was reduced to $47.1 \pm 4.1\%$ of control ($P < 0.001; n = 4$) and amplitude to $80.7 \pm 2.4\%$ of control ($P < 0.05; n = 3$) with one cell showing no change in amplitude.

**Group III mGluR activation: selective decrease in SFO-evoked EPSCs**

None of 19 MnPO neurons exposed to the specific group III mGluR agonist 1-AP4 (50 $\mu$M) demonstrated a significant

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**FIG. 2.** Group I mGluRs selectively suppress subfornical organ (SFO)-evoked excitatory postsynaptic currents (EPSCs) and not inhibitory postsynaptic currents (IPSCs). A: sample traces (average of 5 sweeps) illustrate DHPG-induced (10 $\mu$M, 120 s) reduction in SFO-evoked EPSCs (left): SFO stimulation indicated by $-\rightarrow$ and not IPSCs (right). B: average data illustrate a reversible reduction in amplitude of SFO-evoked EPSCs ($\bullet$, $n = 7$) and not IPSCs ($\cdot$, $n = 9$). SFO-evoked postsynaptic currents (PSCs) were normalized against the average of the 1st 6 PSCs. C: summary histograms exemplify DHPG-induced reduction of SFO-evoked EPSCs (left) and not IPSCs (right). $**$: significant reduction in amplitude of SFO-evoked EPSCs (paired t-test; $P < 0.01$). Note that SFO-evoked IPSCs were recorded as outward currents at $V_m$ $-45$ mV.

**TABLE 1.** Summary of MnPO neurons responding to agonist administration.

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<th>Group</th>
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<th>DCG IV (%)</th>
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**TABLE 2.** Summary of MnPO neurons responding to agonist administration.

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change in either holding current (0.6 ± 0.4 pA) or membrane conductance (103.1 ± 1.1%; \(P > 0.05\)), implying an absence of postsynaptic receptors. When tested for an effect on SFO-evoked currents, L-AP4 induced a partly reversible reduction in the amplitude of SFO-evoked EPSCs (to 51.9 ± 8.4% control; \(P < 0.05\), but lacked any effect on SFO-evoked IPSCs (Fig. 5, A and B). Prior application of (RS)-a-cyclopropyl-4-phosphonophenylglycine (CPPG; 50 \(\mu M\)), a specific group III antagonist, blocked the depressant actions of L-AP4 to 99.8 ± 0.4% of control (Fig. 5C; \(P < 0.05\) ; \(n = 3\)).

In the presence of L-AP4, sEPSCs displayed a significant reduction in frequency (49.3 ± 5.3% control; \(P < 0.05\)) without a change in amplitude (106.6 ± 4% control; \(n = 4\); \(P > 0.05\)). L-AP4 failed to significantly modify sIPSC frequency (91 ± 6% of control) or amplitude (95 ± 2% of control) in either of two cells tested.

When we compared the mGlur agonist-induced reductions from each group on the amplitude of SFO-evoked EPSCs in MnPO neurons, reductions with DCG IV (40.5 ± 5.3% of control) and t-AP4 (45.8 ± 6.2% of control) were significantly larger than that achieved with DHPG (76.7 ± 6.1% of control; \(n = 4\); 1-way ANOVA; \(P < 0.05\)).

Activation of mGlur Rs in MnPO involves presynaptic mechanisms

We next explored whether paired-pulse protocols might provide information as to possible presynaptic mGlur Rs. The data from P2/P1 ratios of SFO-evoked postsynaptic currents reflect diversity in direction as well as magnitude of response. For pharmacologically isolated glutamate ionotropic receptor-mediated EPSCs, 11 cells displayed paired-pulse depression (PPD; 0.78 ± 0.04; \(P < 0.01\)), 11 others displayed paired-pulse facilitation (PPF; 1.23 ± 0.06; \(P < 0.001\)), and 2 cells showed little change (0.99 ± 0.04). As illustrated in Fig. 6, A and B, bath application of agonists for all three mGlur groups significantly increased paired-pulse ratios largely due to the suppression of the first versus the second SFO-evoked EPSC (EPSC\(_1\) = 59.2 ± 4.2% vs. EPSC\(_2\) = 73.1 ± 4.7%; \(P < 0.001\); \(n = 24\)). A lack of significant correlation between agonist-induced conductance and paired-pulse ratio changes (Pearson correlation; \(R = 0.026\); \(P = 0.905\); \(n = 24\)) suggests that mGlurRs for all three groups are present on glutamatergic afferents from the SFO.

Paired-pulse ratios for pharmacologically isolated SFO-evoked IPSCs (200-ms interval) also demonstrated variable ratios: 15 cells displayed PPD (0.82 ± 0.03; \(P < 0.001\)); 6 cells showed weak PPF (1.10 ± 0.01; \(P < 0.05\)); 8 approximated unity (0.98 ± 0.02). As illustrated in Fig. 6, C and D, DCG IV induced a significant change in paired-pulse ratios largely due to suppression in IPSC\(_1\) amplitude relative to IPSC\(_2\) (66.5 ± 4.5% vs. 73.7 ± 4.4%; \(P < 0.001\); \(n = 14\)). Similar to data with EPSCs, there was no correlation between

![Fig. 3](http://jn.physiology.org/). Activation of group II mGlurRs depress MnPO neuronal firing and induce an outward current. A: trace of a cell-attached patch-clamp recording from a MnPO neuron illustrates reversible suppression in firing in response to bath application of a specific group II mGlur agonist DCG IV. Summary histogram of data for 3 MnPO neurons indicates a significant decrease in action potential discharges following application of DCG IV (10 \(\mu M\) for 2–3 min; \(*\), paired \(t\)-test; \(P < 0.05\)). B: whole cell voltage-clamp trace from another MnPO neuron illustrates a slowly rising and reversible outward current in response to bath applied DCG IV (10 \(\mu M\) for 2–3 min; \#), in ACSF containing 1 \(\mu M\) TTX. C: summary histogram of data for 7 MnPO neurons indicates a significant decrease in DCG IV-induced outward current in the presence of a specific group II mGlur antagonist LY 341495 (LY; 100 \(\mu M\) for 5–10 min). D: summary histogram of data for 27 MnPO neurons illustrates a significant increase in membrane conductance during DCG IV-induced outward current. *** significant conductance increase when comparing conductances before (control) and during DCG IV application (paired \(t\)-test; \(P < 0.001\)). E: corresponding current responses to a series of voltage pulses (duration: 600 ms, from −110 to −50 mV with 10-mV steps) applied before (control) and at the peak (DCG IV) of the DCG IV-induced response. I-V plots constructed from values taken at \(\circ\) and \(\bullet\). The net DCG IV-induced current (\(\Delta\)) determined by subtraction of the I-V values displays a reversal potential approximately −90mV.
DCG IV-induced conductance increase and the IPSCs paired-pulse ratio increase (Pearson correlation; \( R = 0.223; P = 0.443; n = 14 \)), suggesting a presynaptic location of group II mGluRs on GABAergic afferents from the SFO. By contrast with data for EPSCs, DHPG and L-AP4 failed to modify SFO-evoked IPSC paired-pulse ratios (Fig. 6D), implying that GABAergic afferents to MnPO arising from SFO neurons lack group I and III mGluRs.

**DISCUSSION**

Currently there are eight known subtypes of G-protein-coupled mGluRs, and these have been classified into three groups (I–III) based on sequence homology, transduction mechanisms, and pharmacological profile (Anwyl 1999; Pinheiro and Mulle 2008; Schoepp 2001). mGluRs have a widespread yet selective distribution in CNS, including the hypothalamic preoptic area where we now report their influence on neuronal excitability in MnPO neurons and on rapid SFO-evoked ionotropic glutamate and GABAA receptor-mediated neurotransmission. The schematics in Fig. 8 are intended to summarize our observations based on responses to exogenous application of select group I–III mGluR agonists and/or antag-

These observations may indicate that the ambient levels of endogenously released glutamate are sufficient to activate heterosynaptic presynaptic group II mGluRs that can reduce the SFO-derived GABAergic afferent drive on MnPO neurons. An alternative explanation is the possibility these receptors are constitutively active.

**Metabotropic GluR antagonists modulate SFO-evoked inhibition**

We next examined whether application of specific antagonists alone might influence resting membrane conductances or SFO-evoked currents. In the small sample of neurons tested, we noted that only the group II mGluR antagonist LY 341495 significantly increased the SFO-evoked IPSCs (Fig. 7, A and B) without a change in resting membrane conductance (102.6 ± 1.5%; \( P > 0.05; n = 3 \)). None of the antagonists induced significant changes in SFO-evoked EPSC amplitudes (Fig. 7B).

**FIG. 5.** Group III mGluRs selectively suppress SFO-evoked EPSCs. A: sample traces (average of 5 sweeps) illustrate that a selective group III mGluR agonist L-AP4 (50 \( \mu M \), 120 s) induces a reduction in SFO-evoked EPSCs (left, SFO stimulation indicated by \( \rightarrow \)) but not IPSCs (right). \( * \) and \( ** \), significant reduction in amplitude of SFO-evoked EPSCs (paired \( t \)-test; \( P < 0.01 \)) and IPSCs (paired \( t \)-test; \( P < 0.001 \)). Note that SFO-evoked IPSCs were recorded as outward currents at \( V_m = -45 \) mV.
onists. Post and presynaptic mGluRs appear to influence the neural circuitry along the upper lamina terminalis in at least two ways. First, >50% of MnPO neurons tested appeared to contain postsynaptic group I mGluRs that enhance neuronal excitability or postsynaptic group II mGluRs that depress neuronal excitability. None of the cells tested exhibited any postsynaptic response to a group III mGluR agonist. Second, agonists for all three mGluR groups were seen to suppress SFO-evoked EPSCs, whereas only a group II mGluR agonist induced suppression of SFO-evoked IPSCs; these actions are likely mediated via a presynaptic mechanism that may involve receptors on SFO afferents. The data from observations using antagonists alone suggest a third possibility, that group II mGluRs may be constitutively active and/or respond to ambient levels of glutamate and thereby attenuate SFO-induced GABAergic neurotransmission in MnPO neurons.

**Postsynaptic effects generated by mGluR groups**

The increase in excitability and TTX-resistant inward current induced in MnPO neurons by DHPG is consistent with mediation via postsynaptic group I mGluRs. Voltage-clamp analysis revealing a net DHPG-induced inward current that was coupled with a decrease in resting membrane conductance that reversed close to −100 mV implies closure of potassium channels. Group I mGluR-induced suppression of resting K⁺ channels has been noted in several CNS sites, including caudate-putamen cholinergic neurons (Takeshita et al. 1996) and hippocampal CA3 neurons (Guérineau et al. 1994). However, voltage-clamp analysis of the DHPG response in a subpopulation of MnPO neurons yielded a net inward current coupled with a conductance increase that reversed around −40 mV or a net DHPG current coupled with no obvious change in membrane conductances and no reversal in the voltage range tested. This suggests that postsynaptic group I mGluRs may couple with other conductances, including nonelective cationic conductances (e.g., Congar et al. 1997; Greene et al. 1994) or cationic currents through activation of the Na⁺-Ca²⁺ exchanger (see Anwyl 1999 for review). Given that the DHPG-induced conductance profile bears a resemblance to that induced by activation of orexin receptors in MnPO (Kolaj et al. 2008), it may interesting in future studies to evaluate a possible relationship between orexin and group I mGluR function.

**FIG. 6.** mGluR agonists modulate SFO-evoked paired-pulse ratios. A: sample traces of paired SFO-evoked EPSCs (interval: 200 ms; SFO stimulation indicated by arrows; average of 5 sweeps) reveal that paired-pulse depression observed in control conditions (gray trace) changes to modest paired-pulse facilitation during the application of DCG IV (5 μM; 120 s; black trace). B: summary histogram illustrates a significant increase in SFO-evoked EPSC paired-pulse ratios for all 3 groups of mGluRs. C: sample traces of paired SFO-evoked IPSCs (interval: 200 ms) reveal that PPD observed in control conditions (gray trace) is eliminated in the presence of DCG IV (5 μM; 120 s; black trace). D: summary histogram illustrates, contrary to SFO-evoked EPSC paired-pulse ratios, a significant increase in SFO-evoked IPSC paired-pulse ratios for mGluR group II and not groups I and III. Single, double, triple asterisks: significant increase in paired-pulse ratios of either SFO-evoked EPSCs or IPSCs (paired t-test; P < 0.05; P < 0.01; P < 0.001, respectively).

**FIG. 7.** An mGluR antagonist alone can modulate SFO-evoked responses. A: sample traces where application of a specific group II mGluRs antagonist LY 341495 (LY; 100 μM for 5–10 min) alone is associated with an increase in amplitude of SFO-evoked IPSCs (SFO stimulation indicated by arrow). B: summary histogram for all cells reveals that mGluR antagonists selectively modified SFO-evoked IPSC but not EPSC amplitudes. Numbers of cells in each category are indicated in bar columns. *, significant increase in amplitude of SFO-evoked IPSCs (paired t-test; P < 0.05).
convergence of signaling mechanisms. DHPG-induced inward current was partly blocked by AIDA at concentrations (500 μM) that should antagonize both mGluR1 and mGluR5 subtypes of group I mGluRs (Moroni et al. 1997). The observation that the mGluR1 preferring CPCCOEt was equally potent in antagonizing the DHPG-induced current would suggest an involvement of both mGluR1 and mGluR5 subtypes in mediating the DHPG-induced effects in MnPO neurons.

By contrast, the response to the group II mGluR agonist DCG-IV was a cessation in firing and reduction in neuronal excitability coincident with a TTX-resistant outward current coupled with increase in resting conductance, blockable by a selective group II mGluR antagonist LY 341495. Voltage-clamp analysis yielding a reversal potential around −96 mV implies opening of K⁺ channels, results that resemble the increase in K⁺ conductances induced by group II mGluR agonists in cerebellar neurons (Knoflach and Kemp 1998) and cochlear Golgi cells (Irie et al. 2006). It is notable that the response to activation of group II mGluRs resembles the postsynaptic changes that follow activation of GABA<sub>B</sub> receptors in MnPO (Kolaj et al. 2004).

Although postsynaptic responses have been reported with group III mGluRs agonists in some CNS sites (e.g., Martin et al. 1997), the lack of any postsynaptic action among MnPO neurons to application of the mGluR group III specific agonist t-AP4 is consistent with results obtained in other brain regions (e.g., Acuna-Goycolea et al. 2003; Matsui and Kita 2003).

Presynaptic modulation of SFO-evoked EPSCs by mGluRs

The present analysis revealed that application of selective agonists for all mGluR groups could suppress SFO-evoked EPSCs in all MnPO neurons. In addition, these effects of DHPG, DCG IV, and t-AP4 on EPSCs were significantly reduced in the presence of specific antagonists AIDA, LY 341495, and CPPG, respectively. In many brain regions, activation of mGluRs, in particular groups II and III, can result in inhibition of glutamatergic transmission (for review, see Anwyl 1999). Our data corroborate this image with group I being significantly less efficient than group II and III, and a delayed or lack of full recovery for response to these two groups of mGluRs could be related to their role in long-term depression (Anwyl 1999).

Although not directly testable, the suppression of SFO-evoked glutamatergic synaptic transmission by agonists for all three groups of mGluRs is likely to involve reduction in the probability of transmitter release from glutamatergic terminals within the MnPO. As noted in other CNS sites, an inhibition of evoked responses was associated with relative facilitation (less inhibition) of the second EPSC of paired EPSCs (PPF) for all three mGluR groups (group I, Fitzjohn et al. 2001; group II, Zheng and Johnson 2003; group III, Matsui and Kita 2003). Second, tests in a subpopulation of MnPO neurons revealed that DHPG, DCG IV, and t-AP4 all prompted a drop in spontaneous EPSC frequencies without effect on their amplitudes. Third, the efficiency of mGluR agonists to decrease amplitudes of EPSCs contrasted with their relatively mild influence on input conductances and holding currents. A lack of correlation between conductance changes and decrease of EPSC amplitudes strongly suggest a heterogeneous distribution of mGluR groups between postsynaptic sites and presynaptic SFO-evoked glutamatergic terminals (Fig. 8). In those instances where both DHPG- and DCG IV-induced frequency changes were coupled with significant changes in spontaneous EPSC amplitudes, one interpretation of such events could be DHPG-induced depolarization and DCG IV-induced hyperpolarization (and subsequent changes in firing patterns) of glutamatergic neurons that reside within the slice preparation and the axons of which project to the MnPO neurons under investigation. One possible source of such modulation is in the SFO itself where stimulation of group II mGluRs with DCG IV was seen to decrease spontaneous IPSCs (Lee et al. 2001). Therefore the collective observations support both direct and indirect actions of mGluRs to modulate neuronal excitability in MnPO neurons in this preparation.

Presynaptic depression of SFO-evoked IPSCs by mGluR group II

Although all three mGluR groups significantly reduced SFO-evoked IPSCs, only application of a group II mGluR agonist resulted in a reduction in amplitude of SFO-evoked IPSCs. DCG IV also increased the paired-pulse ratio due in large measure to less suppression of the second IPSC. This could imply that DCG IV was acting presynaptically to suppress GABA release. Such a conclusion would agree with...
previous studies into control of IPSCs by mGluR group II (Chen and Bonham 2005; Neale and Salt 2006). Effects of mGluR group II activation on SFO-evoked IPSCs resemble those induced by activation of GABA_B and alpha_2 adrenergic receptors in MnPO (Kolaj and Renaud 2007; Kolaj et al. 2004). The observation that DCG IV decreased both the frequency and amplitude of spontaneous IPSCs is consistent with modulation of GABAergic neurons that are located within the slice preparation and the axons of which project to the MnPO neurons under investigation. The glutamate-GABA interaction described here implies that glutamate acting via mGluRs can control not only its own release but also influence SFO-evoked GABAergic inputs.

Functional implications

A survey of the reported expression of mRNA and immunocytochemical data for the different subgroups of mGluRs in the preoptic region reflects considerable variability between groups as well as developmental changes (e.g., Mateos et al. 1998; Ohishi et al. 1995; Shigemoto et al. 1992; van den Pol et al. 1995). While the SFO and MnPO have been implicated in a variety of homeostatic functions that include feeding, sleep-waking behaviors, hydromineral, cardiovascular, and thermal regulation, few studies have attempted to address the roles of mGluRs within this specific brain region (e.g., Yamaguchi and Watanabe 2004). Ascertaining the specific roles of the different groups of mGluRs within this region will need to incorporate the various elements of the neuronal circuitry along the upper lamina terminalis. Therefore the present investigation represents an initial approach at the cellular level to focus on how the different types of mGluRs might attenuate MnPO neuronal excitability and their glutamatergic and GABAergic inputs that are deemed to arise from SFO neurons.

From a postsynaptic perspective, the present data indicate that activation of group I or II mGluRs will induce contrasting effects on MnPO excitability, the former excitatory, the latter inhibitory, thus their ability to regulate patterns of activity. This may be of particular importance among subpopulations of MnPO neurons that possess calcium-dependent rhythmic activity through activation of T-type low-voltage-activated calcium channels (Spanswick and Renaud 2005). Thus group I mGluRs might contribute to inactivation of these T-type Ca^{2+} channels and tonic firing as occurs normally during membrane depolarization, whereas group II mGluR-induced membrane hyperpolarization might be a factor in removing or de-inactivating these T-type Ca^{2+} channels and the promotion of burst firing. Such changes in firing pattern will convey distinctly different messages at sites targeted by these MnPO neurons.

Because the MnPO contains both glutamatergic and GABAergic neurons, one might anticipate that future investigations where the phenotype of the neurons under study is revealed might help to clarify roles for mGluRs. For instance, recent functional studies have implied a differential regulation that such GABAergic MnPO neurons are more active during sleep, whereas glutamatergic neurons are more active during systemic hyperosmotic challenges and following intracerebroventricular angiotensin (Grob et al. 2003; Gvilia et al. 2005). While the participation of postsynaptic group I or II mGluRs in these situations remains to be defined, one might hypothesize that postsynaptic mGluRs participate in the MnPO-mediated inhibitory control over arousal-related and excitatory control over sleep-related perifornical/lateral hypothalamic neurons (see Suntsova et al. 2007). From a presynaptic perspective, the ability of mGluRs to attenuate SFO-evoked responses in MnPO implies their contribution in modulating inputs to MnPO. A challenge in future investigations will be to decipher more precisely the nature and impact of the various presynaptic mGluRs on afferents to MnPO neurons. On the assumption that the SFO innervation provides MnPO neurons with information related to the roles attributed to this particular sensory circumventricular organ, a schematic portrait (Fig. 8) of the involvement of the different groups of mGluRs in this circuitry may be useful in the design of investigations that will elaborate in more detail how mGluRs participate in neurotransmission along the upper lamina terminalis.

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