Role of metabotropic glutamate receptors in vasopressin and oxytocin release

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Morsette, Delmore J., Hanna Sidorowicz, and Celia D. Sladek. Role of metabotropic glutamate receptors in vasopressin and oxytocin release. Am J Physiol Regulatory Integrative Comp Physiol 281: R452–R458, 2001.—The effect of metabotropic glutamate receptor (mGluR) activation on vasopressin (VP) and oxytocin (OT) release was evaluated using explants of the hypothalamoneurohypophysial system. (+/−)-1-Aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD), an agonist at groups I and II mGluRs, increased VP and OT release in a concentration-dependent manner. A role for group I mGluRs in VP and OT release was demonstrated by the ability of a group I-specific mGluR antagonist, 1-amino-1,5-idanecarboxylic acid (AIDA), to block the effect of t-ACPD and the ability of a group I-specific agonist, (R,S)-3,5-dihydroxyphenylglycine, to significantly increase both VP (P = 0.0029) and OT (P = 0.0032) release. However, AIDA did not alter VP or OT release induced by a ramp increase in osmolality of the perfusion medium. The role of group III mGluRs was examined using L(+)-2-amino-4-phosphonobutyric acid (L-AP4), an agonist of these receptors. L-AP4 did not change basal release of VP or OT and did not prevent osmotically stimulated hormone release. Thus mGluR activation stimulates VP and OT release, but it is not required for osmotic stimulation of hormone release.

supraoptic nucleus; neurohypophysis; excitatory amino acids; osmotic stimulation

The evidence for postsynaptic mGluRs on the SON neurons themselves derives from whole cell patch-clamp and intracellular recording data (11). Application of trans-1-amino-1,3-cyclopentane dicarboxylic acid (t-ACPD), a group I and II mGluR agonist, resulted in an inward current, a slow depolarization, and a decrease in conductance in one-half of the magnocellular neurons (11). A group I and II mGluR antagonist, (R,S)-α-methyl-4-carboxyphenylglycine, blocked these effects of t-ACPD. Both a specific group II-receptor agonist, 2S,1′,2′S-2-carboxycyclopropylglycine, and a group III agonist, L(+)-2-amino-4-phosphonobutyric acid (L-AP4), did not alter these parameters (11). The group I mGluRs on SON neurons mediate their effects by reducing the resting voltage-gated and Ca2+-activated K+ currents (21). The evidence points toward the presence of a group I mGluR on the magnocellular neurons and supports the immunohistochemical evidence of mGluR1b receptors in the SON (6).

Presynaptic effects of groups I and III mGluRs have been reported in the SON. The application of L-AP4, a group III-specific agonist, decreased the frequency of excitatory postsynaptic currents and inhibitory postsynaptic currents in the SON indicative of inhibition of both GABA and glutamate release from presynaptic terminals (11, 21). Similarly, t-ACPD and (R,S)-3,5-dihydroxyphenylglycine (DHPG), a group I mGluR-specific agonist, increased the frequency of both EPSCs and IPSCs in both vasopressin (VP) and oxytocin (OT) neurons. However, this effect was inhibited in the presence of tetrodotoxin (TTX), indicating a presynaptic location on the somas of local inhibitory, GABAergic, and excitatory glutamatergic neurons adjacent to the SON that innervate magnocellular neurons (1, 2, 11, 21). Thus neurons afferent to the SON neurons express multiple mGluRs that are differentially located. Specifically, mGluR group I receptors, possibly mGluR1, are on the somata and/or dendrites of local neurons that innervate the SON, and mGluR group III receptors, possibly mGluR7, are located on the terminals of these same neurons. Both of these hypotheses have been demonstrated to be present in the hypothalamus and are expressed in the SON (4–6).

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In the current studies, various mGluR agonists and antagonists were evaluated for effects on VP and OT release from explants of the hypothalamoneurohypophysial system (HNS). The purpose was to extend the prior electrophysiological observations by examining the role of mGluRs in neuropeptide hormone release. The effects of t-ACPD (a group I and II mGluR agonist), DHPG (a specific group I agonist), and L-AP4 (an mGluR group III agonist) were evaluated. In addition, a selective group I antagonist, (R,S)-1-aminoindo- dan-1,5-dicarboxylic acid/ UPF523 (AIDA), and L-AP4 were used to examine the possible involvement of mGluR groups I and III receptors in osmotic stimulation of VP and OT release.

METHODS

Preparation and perifusion of HNS explants. HNS explants were obtained from decapitated male Sprague-Dawley rats (125–150 g). The explants included the OT and VP magnocellular neurons of the SON with their axonal projections extending through the median eminence and terminating in the neural lobe. The explants also included the organum vasculosum of the lamina terminalis (OVLT), the suprachiasmatic nucleus, and the arcuate nucleus. To prepare the explant, the brain was removed from the skull using a caudal approach to maintain the pituitary stalk intact. Under a dissecting microscope, the anterior pituitary was removed along with the dura mater and arachnoid layers. A block of tissue was removed by cutting rostral to the optic tracts, lateral to the median eminence, and caudal to the pituitary stalk. The explant is ~1–2 mm thick, and at the rostral end it is ~5-mm wide at its widest point.

HNS explants were perifused at 37°C with oxygenated culture medium. The explants were placed in closed chambers (0.5 ml) and perifused at a rate of 1.8–3.0 ml/h. The medium used was F12 nutrient mixture (GIBCO or Sigma). The medium was fortified with 20% fetal bovine serum, 1 mg/ml glucose, 50 U/ml penicillin, 50 μg/μl streptomycin, and 0.1 μM bacitracin. Bacitracin was used to prevent degradation of the OT and VP in the collected medium. Six explants were perifused simultaneously during each experiment. The medium from each chamber was collected at 5°C in a refrigerated fraction collector and stored at −20°C until extracted for RNA.

Experimental procedures. The explants were perifused for 4 h before application of any pharmacological agent or change in osmolality to allow the explant to equilibrate and to establish a basal rate of hormone release. Subsequent hormone release was normalized to the basal release of that explant at the end of the equilibration period and is expressed as a percentage of this basal value. The change in osmolality to allow the explant to equilibrate and to establish a basal rate of hormone release was normalized to the basal release of that explant at the end of the equilibration period and is expressed as a percentage of this basal value.

When explants were exposed to increased osmolality, the osmolality of the perifusate was increased over a 6-h period by 20–40 mosmol/kgH2O with a ramp increase in NaCl. NaCl was chosen as the osmotic agent because physiological changes in osmolality of the extracellular fluid primarily reflect changes in sodium. Although sodium may elicit specific responses, HNS explants have been shown to respond to increases in osmolality that are sodium independent (17). Therefore, the response to the ramp increase in NaCl cannot be considered as sodium specific, and thus it is referred to as an osmotic stimulus.

Explants were exposed to the following mGluR agonists: t-ACPD (5–200 μM; Tocris), DHGP (50 μM; Tocris), and L-AP4 (50–300 μM; Tocris). AIDA, a group I mGluR antagonist (300 μM; Tocris), was also used. With the exception of DHGP, which is water soluble, stock solutions of these agents were prepared in 1 eq NaOH and then diluted into perifusion medium. A comparable amount of base was added to the medium for the control explants. This did not result in a noticeable change in pH (F12 medium contains phenol red as a pH indicator).

Radioimmunoassay. VP and OT concentrations in the perifusate fractions were determined by radioimmunoassay as described previously (19). The antisera used were generated in conjunction with Arnel Products (Brooklyn, NY). The antibodies were used at final dilutions of 1:100,000–1:1,500,000. Duplicate 50- and 100-μl aliquots of each fraction were assayed. All samples from a given experiment were assayed at the same time. The minimum sensitivity was 1.0 pg/tube for VP and 0.5 pg/tube for OT.

RNA extraction and quantification. VP and OT mRNA content of each explant at the end of the perifusion period was determined by ribonuclease protection assay as described previously (20). A 32P-labeled full-length VP cRNA probe (680 bp) synthesized using the Promega Riboprotein System from the pGEM 4-VP8C construct provided by T. G. Sherman, Georgetown University, was used in the assay. VP and OT mRNA could be assayed simultaneously, because the full-length VP mRNA probe includes the region of exon II that is homologous between VP and OT mRNA. This region codes a portion of neurophysin. Thus the protected fragments from VP and OT mRNA were different sizes (e.g., 680 vs. 200 bp, respectively) and could be separated on 1.3% agarose gel. Total extracted RNA from each explant was used in the hybridization.

VP and OT mRNA content was determined by counting the radioactivity in gel fragments containing the VP and OT mRNA bands. Standards of 0, 50, 100, 500, and 1,000 pg of sense VP mRNA generated from the cAVP-8C plasmid as well as RNA from a nonperifused explant and standard preparations of RNA from hydrated and dehydrated rats were included on each gel for comparison.

Data analysis. The results are expressed graphically as means of the percent of basal release ± SE. Statistical significance was determined by two-way ANOVA with repeated measures following log10 transformation of the data. When the ANOVA indicated statistical significance, individual mean comparisons were performed by simple effects or Newman-Keuls analysis to examine if the difference at specific time points was significant. The level of significance was set at P ≤ 0.05. On each figure, an arrow or a box indicates the time when the HNS explants were exposed to each agent, F values represent overall group differences, and symbols indicate the probability that hormone release was different between groups at individual time points.

RESULTS

Effect of t-ACPD on hormone release. The role of groups I and II mGluRs in hormone release was evaluated by perifusing explants with t-ACPD at various concentrations for 2 h or longer. A concentration-dependent stimulation of both VP and OT release was observed when the peak response to 5, 12.5, 25, or 100 μM t-ACPD was evaluated (Fig. 1). Both VP and OT releases were significantly increased compared with control explants that received only perifusion medium.
Effect of a group I mGluR antagonist, AIDA, on t-ACPD-induced hormone release. Due to the fact that t-ACPD is an agonist at both group I and group II mGluRs, other agents were used to further elucidate the receptor type responsible for the increase in hormone release. HNS explants were exposed to t-ACPD for 4 h in the presence of the group I mGluR antagonist AIDA. AIDA (300 μM) blocked the t-ACPD-induced (100 μM) increase in both VP and OT release (Fig. 2, A and B). This suggests that the effect of t-ACPD on VP and OT release is mediated through activation of group I mGluRs.

Effect of DHPG, a group I-specific mGluR agonist. To further evaluate the role of group I mGluRs in VP and OT release, a more specific agonist was applied to the HNS explants. Explants were exposed to DHPG (50 μM), an agonist specific for group I mGluRs, for 4 h following the equilibration period. DHPG induced a significant increase in both VP and OT release compared with the control groups (Fig. 3, A and B). This confirms that group I mGluRs increase VP and OT release, and it suggests that this is the receptor type responsible for the effect of t-ACPD.

Effect of AIDA on osmotically stimulated hormone release. To evaluate the role of the group I mGluRs in response to an osmotic stimulus, HNS explants were exposed to a gradual increase in NaCl concentration of 30 mosmol/kgH2O over a 6-h period in the presence and absence of AIDA. AIDA was added immediately before initiating the ramp increase in osmolality. As shown in Fig. 4, A and B, the increase in osmolality significantly stimulated VP and OT release. The effect of the ramp increase in osmolality on VP and OT release was not altered in the presence of AIDA (300 μM). Thus, unlike the ionotropic GluRs (15, 19), group I mGluRs do not appear to be essential for HNS explants to respond to an osmotic challenge. A subset of explants evaluated for osmotic stimulation of hormone in the presence and absence of AIDA (300 μM) was also evaluated for changes in VP and OT mRNA content. AIDA did not significantly alter either VP or OT mRNA (Fig. 5, A–C).

Effect of group III mGluR agonist, L-AP4, on basal and osmotically stimulated hormone release. As described previously, the presynaptic location of the

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**Fig. 1.** Stimulation of vasopressin (VP) and oxytocin (OT) hormone release by (+/-)-1-aminoindan-1,5-idicarboxylic acid (t-ACPD). Both VP and OT release were significantly increased at all concentrations of t-ACPD tested. Basal release: control group 45.9 ± 20 pg/ml; t-ACPD group 49.3 ± 18 pg/ml. OT release was also increased significantly. The release patterns for VP and OT were similar. Basal release: control group 142 ± 42 pg/ml; t-ACPD group 59 ± 15 pg/ml. *Significant difference from control; n = 6–10 per mean.

**Fig. 2.** Effect of 1-aminoindan-1,5-idicarboxylic acid (AIDA; 300 μM) on t-ACPD-induced (100 μM) VP and OT release. A: exposure to 100 μM t-ACPD significantly increased VP release compared with control (F = 11.082, P = 0.0054). t-ACPD-induced VP release was blocked in the presence of AIDA (F = 15.25, P = 0.0016). Basal release: t-ACPD group (n = 10) 87.3 ± 38.0 pg/ml; t-ACPD + AIDA group (n = 6) 271.1 ± 38.0 pg/ml; control group (n = 5) 53.7 ± 32.3 pg/ml. B: OT release induced by 100 μM t-ACPD (F = 23.18, P = 0.0004) was also blocked by AIDA (F = 18.75, P = 0.001). Basal release: t-ACPD group (n = 8) 63.7 ± 9. pg/ml; t-ACPD + AIDA group (n = 6) 96.3 ± 18.3 pg/ml; control group (n = 5) 169.0 ± 47 pg/ml. *P < 0.05 compared with control and t-ACPD+AIDA groups.
group III mGluRs provides the opportunity for these receptors to inhibit excitatory and inhibitory transmission to the VP and OT neurons. Because endogenous GABA release suppresses VP release from HNS explants (14), it was postulated that activation of group III mGluRs would inhibit GABA release resulting in an increase in VP release. To determine if group III mGluRs alter VP and OT release, L-AP4, a group III mGluR agonist, was applied sequentially at 50, 150, and 300 μM at 2-h intervals. There was no significant difference in VP or OT release compared with the control groups (data not shown).

Because glutamatergic afferents transmit information from the osmoreceptors to the VP and OT neurons in the SON, the presynaptic location of group III mGluRs also offers the potential for modulation of osmotic stimulation of hormone release. As in previous experiments, explants were exposed to a ramp increase in osmolality in the presence of L-AP4 (300 μM) compared with the controls. Osmotically stimulated OT release was not significantly different in the presence and absence of L-AP4, but osmotically stimulated VP release was statistically slightly greater in the presence of L-AP4.

**DISCUSSION**

These results demonstrate that the mGluRs are an important part of a complex system for activation of VP release and provide additional support for the notion that glutamatergic afferents from osmoreceptors in the SON control hormone release. The efficacy of L-AP4 in increasing VP release in the presence of osmotically stimulated hormone release suggests that these receptors play a role in mediating osmotic stimulation of VP release. The lack of effect on OT release in the presence of osmolality and L-AP4 suggests that the OT neurons are not as sensitive to changes in glutamatergic transmission. This finding is consistent with previous studies that have suggested a potential role for mGluRs in the regulation of VP release but not OT release.
and OT release by excitatory amino acids. The release results are consistent with previous electrophysiological evidence for mGluR activation of the SON neurons as well as immunohistochemical evidence for receptor expression.

In the SON, t-ACPD has been shown to act postsynaptically both on magnocellular neurons and on afferent neurons that include both GABA and glutamate neurons. Application of 100 \( \mu M \) t-ACPD resulted in increased frequency of excitatory postsynaptic potentials (EPSPs) in 20% of cells recorded and of inhibitory postsynaptic potentials (IPSPs) in 50% of cells recorded. This effect could be blocked by TTX and mimicked by DHPG (50 \( \mu M \)) (12). In addition, t-ACPD also inhibited \( K^+ \) conductance in SON neurons resulting in an inward current and slow depolarization (11). Similar effects have been seen in the hippocampus, dorsolateral septal nucleus, geniculate thalamus, nucleus of the solitary tract, cortex, and cerebellum where t-ACPD and DHPG can both induce slowly developing depolarization and an inward current associated with increases in cell firing (10). The overall effect of t-ACPD is an increase in excitability of the magnocellular neuron.

In the current studies, the observed increase in VP and OT release was probably due to the postsynaptic effects of t-ACPD and DHPG to depolarize magnocellular neurons. If the effect on local afferent neurons

Fig. 5. Effect of AIDA (300 \( \mu M \)) on osmotically induced VP and OT mRNA. A and B: VP and OT mRNA content presented as counts per minute is not altered in the presence of AIDA. C: representative autoradiogram showing a subset of the explants analyzed in A and B. Densely labeled VP (680 bp) and OT (200 bp) bands are present in the standard sample of hypothalamic mRNA from dehydrated rats run simultaneously.

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Fig. 6. Effect of L(+)-2-amino-4-phosphonobutyric acid (L-AP4; 300 \( \mu M \)) on osmotically stimulated VP and OT release. A: L-AP4 did not inhibit VP release in response to Osm; however, VP release was slightly augmented in the Osm + AP4 group compared with Osm. *Time points where these 2 groups were significantly different. There is a significant difference between L-AP4 + Osm group compared with the control group (\( F = 56.040, P < 0.0001 \)). †Time points where L-AP4 + Osm and control groups are significantly different. Basal release: Osm group 49.4 ± 15.6 pg/ml; Osm + L-AP4 group 8.9 ± 3.3 pg/ml; control group 32.2 ± 8.8 pg/ml. B: there was no significant difference in OT release in response to Osm in the presence or absence of L-AP4 (\( F = 2.653, P = 0.1170 \)). There was a significant increase in OT release in the L-AP4 + Osm group compared with the control group (\( F = 27.482, P = 0.0002 \)). Basal release: Osm group 141.3 ± 33.9 pg/ml; Osm + L-AP4 group 85.8 ± 30.3 pg/ml; control group 155.0 ± 43.3 pg/ml.
was dominant, the expectation would be to see an inhibition of release due to the larger number of presynaptic GABAergic terminals vs. glutamatergic in SON (8). This predominance of GABAergic innervation may be responsible for the greater percentage of IPSPs found electrophysiologically. Because the ACPD-induced depolarization of SON neurons is sufficient to elicit action potential firing, it would also be expected to result in an increase in hormone release as was observed in the current experiments. Other possible sites of action of t-ACPD and DHPG include the VP and OT nerve terminals in the posterior pituitary and glial cells in either the hypothalamus or pituitary. Although many agents modulate VP and OT release via direct actions on the nerve terminals in the posterior pituitary (for review, see Ref. 13), significant binding of H3-glutamate to mGluRs was not detected in the neural lobe (7). Thus this site is unlikely to account for the observed effects of t-ACPD via either actions on the terminals or on pituicytes. Expression of group I mGluRs has been reported on hippocampal astrocytes (18), and astrocytes are thought to play a dynamic role in regulating excitability of SON neurons. Therefore, glial involvement in the response to mGluR agonists is an intriguing possibility that could account for differences between electrophysiological and release responses such as the extended nature of the release induced by t-ACPD. However, further studies are required to substantiate the expression of mGluRs by SON astrocytes.

Another difference between electrophysiological and release results was the relative effectiveness of t-ACPD and DHPG on VP compared with OT neurons. In the electrophysiological studies, a preferential effect on VP neurons compared with OT was reported: 80% of VP neurons responded to t-ACPD compared with 33% of identified OT neurons (11). In contrast, VP and OT release in response to either DHPG or t-ACPD was not substantially different.

The blockade of t-ACPD-induced VP and OT release by AIDA adds further support for the peptide release being mediated via activation of group I mGluRs, in particular mGluR1. Although AIDA does act preferentially at mGluR1, the concentration used in the current experiments (300 μM) is borderline for antagonist action at mGluR5 as well (9). However, although mGluR5 expression has been reported in the hypothalamus (23), the strong expression of mGluR1b in the SON (6), the total blockade of t-ACPD-induced VP and OT release by AIDA, and the effect of DHPG support the conclusion that the t-ACPD effect is mediated via mGluR1. Although the possibility remains that a separate role for group II receptors might be demonstrated using a specific group II agonist, no effect of the specific group II agonist 2S,1',2'S-2-carboxycyclopropylglycine was observed electrophysiologically (11).

Recently, the specificity of some compounds considered to be specific agonists or antagonists for mGluRs has been questioned due to evidence that they alter responses of N-methyl-D-aspartate receptors (NMDA-Rs) (3). Specifically, activation of NMDA-Rs by high concentrations of some mGluR compounds probably reflected the presence of amino acid contaminants in the preparation (3). It is unlikely that such contamination accounts for the effects of the mGluR compounds reported in this study for the following reasons. Agonist effects of t-ACPD on NMDA-Rs were seen at 100 μM, but stimulation of VP and OT release was present at much lower concentrations of t-ACPD (5, 12.5, and 25 μM). For L-AP4, glycine contamination was thought to be responsible for NMDA-R agonist activity, but this was observed at a concentration of 1 mM, whereas the maximal amount used in the current experiments was 300 μM. DHPG caused a slight inhibition in NMDA-induced currents, but, in contrast, VP and OT release was increased, not decreased, by DHPG. As for AIDA, NMDA-induced currents were partially attenuated (~25%), but AIDA completely blocked t-ACPD-induced VP and OT release. Furthermore, AIDA did not alter osmotically stimulated VP and OT release, which is blocked by NMDA-R antagonists (19). Thus the possible actions of these compounds on NMDA-Rs do not appear to be a factor in interpreting their effects on hormone release.

As mentioned, osmotically stimulated VP and OT release was not prevented by blocking the postsynaptic group I mGluRs with AIDA or by blocking presynaptic group III mGluRs with L-AP4. Thus, despite the ability of group I mGluR agonists to induce VP and OT release, these receptors are not required for osmotic stimulation. Although it was originally hypothesized that group I mGluRs may enhance or extend the response initiated by ionotropic GluR (iGluR) or regulate gene expression, sustained increases in osmotically stimulated hormone release were evident in the presence of a concentration of AIDA able to block the effects of t-ACPD. Furthermore, the lack of a difference in mRNA content suggests that the osmotically induced increase in VP and OT mRNA was not compromised. Therefore, the data suggest that the ionotropic mechanisms are sufficient both for osmotically induced increases in hormone release and mRNA content. Because glutamate mediates the osmotically induced increases in EPSPs that initiate action potentials in SON neurons and because there is evidence for the group III mGluRs, specifically mGluR7 expression, in the hypothalamus and SON (4), it was hypothesized that group III mGluRs might block osmotic stimulation of hormone release by preventing or attenuating glutamate release. However, this was not observed. Instead, there was a slight augmentation of basal and osmotically induced VP release by L-AP4. This could reflect inhibition of GABA release, because L-AP4 decreased the frequency of IPSPs in magnocellular neurons (12). However, this effect was small and was not evident in osmotically stimulated OT release or in basal release of either VP or OT. Thus these results indicate that the group III mGluRs do not play a major role in modulating either glutamate or GABA release from SON afferents in perfused HNS explants, and they do not interfere with osmotic stimulation of hormone release.
In conclusion, these results indicate a role for the mGluRs in VP and OT release with the postsynaptic activation of group I mGluRs in SON neurons being sufficient to elicit hormone release. Further evaluation of mGluR interactions with iGluRs is warranted to provide a better understanding of the specific role of these receptors in regulation of VP and OT hormone release.

**Perspectives**

VP and OT release from the neural lobe is regulated by a wide variety of neurotransmitters, neuropeptides, and hormones, as well as osmolality of the extracellular fluid. The role of the excitatory amino acid neurotransmitter glutamate in this complex is further complicated by its ability to interact with multiple receptors with both ionotropic and metabotropic signaling characteristics. The current study focused on the metabotropic class of these receptors and demonstrated stimulation of VP and OT release by a single group of mGluRs. It is significant that these receptors do not appear to participate in osmotic regulation of VP and OT release, because these responses are dependent on activation of ionotropic glutamate receptors. Also, other G protein-linked neuropeptide receptors have been shown to modulate osmotic stimulation of VP release (16). Thus the response is not mediated solely by ionotropic responses.

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