Vasopressin and Amastatin Induce V₁-Receptor-Mediated Suppression of Excitatory Transmission in the Rat Parabrachial Nucleus

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Chen, Xihua and Quentin J. Pittman. Vasopressin and amastatin induce V₁-receptor-mediated suppression of excitatory transmission in the rat parabrachial nucleus. J. Neurophysiol. 82: 1689–1696, 1999. We examined actions of arginine vasopressin (AVP) and amastatin (an inhibitor of the aminopeptidase that cleaves AVP) on synaptic currents in slices of rat parabrachial nucleus using the nystatin-perforated patch recording technique. AVP reversibly decreased the amplitude of the evoked, glutamate-mediated, excitatory postsynaptic current (EPSC) with an increase in paired-pulse ratio. No apparent changes in postsynaptic membrane properties were revealed by ramp protocols, and the inward current induced by a brief application of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid was unchanged after AVP. The reduction induced by 1 μM AVP could be blocked by a V₁ AVP receptor antagonist, [d(CH₂)₅Arg₈]-vasopressin (Manning compound, 10 μM). Bath application of amastatin (10 μM), reduced the evoked EPSC, and AVP induced further synaptic depression in the presence of amastatin. Amastatin’s effects also could be antagonized by the Manning compound. Corticotropin-releasing hormone slightly increased the EPSC at 1 μM, and coapplication with AVP attenuated the AVP response. Pretreatment of slices with 1 μg/ml cholera toxin or 0.5 μg/ml pertussis toxin for 20 h did not significantly affect AVP’s synaptic action. The results suggest that AVP has suppressant effects on glutamatergic transmission by acting at V₁ AVP receptors, possibly through a presynaptic mechanism involving a pertussis-toxin- and cholera-toxin-resistant pathway.

INTRODUCTION

Arginine vasopressin (AVP) has profound effects in the CNS on behavior, body temperature, and cardiovascular regulation, sympathetic and neuroendocrine activation, and cognition (for reviews, see de Wied et al. 1989, 1993; Herbert 1993; Pittman et al. 1998; Swanson and Sawchenko 1980). These effects are mediated mainly by the V₁-type AVP receptors in keeping with observations that the V₂ subtype is most abundant in the brain (Ostrowski et al. 1992; Tribollet et al. 1988). Distinctive AVP cell groups have been identified to regulate central functions with, for example, the paraventricular nucleus activating the neuroendocrine and autonomic systems (Antoni 1993; Plotsky 1991) and the bed nucleus of stria terminalis promoting antipyresis (Pittman and Thornhill 1990; Pittman et al. 1998). With respect to possible regulation of autonomic function, it is demonstrated that a reciprocal innervation exists between various hypothalamic nuclei and the parabrachial nucleus (PBN) in the pons (Jhamandas et al. 1991; Krukoff et al. 1992; Moga et al. 1990). In keeping with its role in relaying sensory and visceral information to higher centers, the PBN has been implicated in cardiovascular regulation. Stimulation of the baroreceptor afferents causes alteration in glutamate release in the PBN (Saleh et al. 1997a) and stimulation of the PBN causes a pressor response (Chamberlin and Saper 1992; Sved 1986) attributable to increased AVP release from the hypothalamus (Sved 1986).

As AVP is thought to act as a central neurotransmitter involved in cardiovascular regulation (Pittman and Bagdan 1992) and AVP immunoreactive fibers have been detected in the PBN (Block and Hoffman 1987), we were interested in defining the cellular action of AVP in this nucleus. In the brain, AVP-induced direct excitation of the postsynaptic cells has been observed in the suprachiasmatic nucleus, amygda. area postrema, facial, and dorsal vagal motorneurons (Ingram and Tolchard 1994; Lowes et al. 1995; Lu et al. 1997; Mihai et al. 1994; Mo et al. 1992; Widmer et al. 1992). AVP also has been found to increase excitatory synaptic transmission in a number of brain regions including the hippocampus and the dorsolateral septum (Chen and Du 1993; Chepkova et al. 1995; Miura et al. 1997; Van den Hooff and Urban 1990; Van den Hooff et al. 1989). In the hippocampus, however, it has been reported that excitation of pyramidal cells is reduced due to AVP’s primary excitation of the local circuit inhibitory interneurons (Albeck et al. 1990; Smock et al. 1990). Recently work from our lab demonstrated that AVP reduces the evoked excitatory postsynaptic current (EPSC) in the magnocellular cells of the supraoptic nucleus without affecting the postsynaptic membrane properties (Mougnot et al. 1998). Regardless of pre- or postsynaptic site of action, these reports concur that V₁ receptors are implicated because of effective blockade by the V₁ antagonist. V₂-receptor-mediated effects, although not as commonly seen as V₁-mediated ones, have been observed on acutely dissociated diagonal band of Broca cells (Easaw et al. 1997).

Corticotropin-releasing hormone (CRH) is another neurotransmitter implicated in central cardiovascular regulation (Fisher 1993), and the PBN has intrinsic CRH-containing neurons and also receives an extrinsic CRH innervation (Merchanthaler et al. 1982; Otake and Nakamura 1995; Palkovits et al. 1985). CRH has been found to increase spontaneous discharge rate of locus coeruleus cells in vivo (Borsody and Weiss 1996) and excite dissociated or cultured neurons in vitro (Aldenhoff et al. 1983; Fox and Grul 1993). CRH-induced excitation is reflected by its actions on
calcium and potassium channels. It is reported that CRH increases whole cell Ca\(^{2+}\) currents in dissociated amygdaloid cells (Yu and Shinnick-Gallagher 1998) and intracellular Ca\(^{2+}\) in corticotrophs (Lee and Tso 1997), and decreases K\(^+\) conductances in hippocampal and cerebellar Purkinje cells (Aldenhoff et al. 1983; Fox and Grudz 1993).

Neurons that coexpress AVP and CRH show a remarkable plasticity in the synthesis and release of these peptides (Sawchenko 1987), and these peptides exert synergistic actions on anterior pituitary cells where their receptors are colocalized (Antoni 1993; Leong 1988). In light of similar distribution of fibers immunoreactive for AVP and CRH in the PBN, we therefore determined if there is a similar interaction between CRH and AVP in terms of synaptic modulation.

This work was performed to determine: whether AVP modulates excitatory transmission in the PBN and, if so, which type of receptors are involved; whether AVP’s action is accentuated by an aminopeptidase inhibitor that prevents AVP cleavage; whether AVP interacts with CRH; and whether alterations in G-protein activity change AVP’s synaptic action.

METHODS

Slice preparation

Male Sprague-Dawley rats (180–220 g) were anesthetized with halothane. The brain was removed quickly and placed into ice-cold, carbogenated (95% O\(_2\)-5% CO\(_2\)) artificial cerebrospinal fluid (ACSF; pH 7.3–7.4). Coronal slices (400-μm thick) were cut from a block of tissue containing the PBN (bregma −9.1 to −9.8 mm (Paxinos and Watson 1986)) in cold (4°C) carbogenated ACSF using a vibratome. Slices were hemisected and incubated in ACSF at room temperature (22°C) for ≥1 h before recording. A slice then was transferred into a 500-μl recording chamber where it was submerged and continuously perfused with prewarmed ACSF (28–30°C) at a rate of 2–3 ml/min. The composition of the ACSF was (in mM) 126 NaCl, 2.5 KCl, 1.2 Na\(_2\)HPO\(_4\), 1.2 MgCl\(_2\), 2.4 CaCl\(_2\), 18 NaHCO\(_3\), and 11 glucose. To eliminate possible GABAergic contamination of excitatory synaptic responses, 50 μM picrotoxin was present in the ACSF at all times.

Nystatin-patch recording

Nystatin patch recordings from PBN neurons were made with glass micropipettes (Garner Glass; tip resistance 4–10 MΩ) first back-filled at the tip with a solution containing (in mM) 120 M K-acetate, 40 HEPES, 5 MgCl\(_2\), and 10 EGTA and then filled with the same solution containing nystatin 450 μg/ml and Pluronic F127 (dissolved in DMSO). High-resistance seals (1–3 GΩ) were made using an Axoclamp 2A amplifier. A −20-mV step (100-ms duration) was applied to monitor the partitioning of nystatin into the membrane. Access to the cell (series resistance of 15–30 MΩ) was attained within 30 min after seal formation.

Data acquisition and analysis

After adequate access was attained, resting membrane potential was measured and I-V characteristics were obtained by current injections (in steps of 10 pA) in the current-clamp mode. All responses were filtered at 1 or 3 kHz. Cells then were voltage clamped near the resting membrane potential at −65 mV (Zidichouski et al. 1996). Synaptic currents were evoked by applying single pulses via a bipolar stimulating electrode placed close to the ventral tip of the superior cerebellar peduncle. A stimulus intensity that yielded a response 50–60% of the maximum synaptic response was used for the remainder of the experiment. Three successive synaptic responses were taken 10 s apart, digitally averaged and stored for off-line analysis. In all synaptic current experiments, a −20-mV, 100-ms square pulse was applied 150 ms after synaptic stimulation to monitor input and series/access resistance. In addition to the computer-assisted data acquisition, continuous records of membrane potentials and currents were made on a pen chart recorder. In toxin-treated slice experiments, slices were hemisected and one-half of them was incubated in either 1 μg/ml cholera toxin (CtxT) or 0.5 μg/ml pertussis toxin (PTX) in a volume of 3 ml ACSF for 18–20 h while the other halves were incubated in carbogenated ACSF of the same volume for the same time period.

All acquired data were analyzed off-line using Clampfit (Axon Instruments, Foster City, CA). Data are expressed as means ± SE in either absolute values or normalized percentages. Statistical comparisons were performed on raw data using paired or unpaired Student’s t-test or analysis of variance (ANOVA) where appropriate. Significance was accepted at the 0.05 level.

All drugs (except for CtxT and PTX) were bath applied by perfusion with ACSF containing the final concentration of the drug. AVP, CRH, Manning compound, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and CtxT were from RBI (Natick, MA); amastatin, nystatin, picrotoxin, and ACSF components were from Sigma (St Louis, MO). Stock solutions of CNQX, AMPA, AVP, and CRH were aliquoted and frozen at −20°C and diluted into ACSF immediately before the experiment.

RESULTS

Patch recordings were made from cells located in both mesencephalic and pontine divisions of the lateral PBN and predominantly from the dorsal lateral and external lateral subnuclei. Cell characteristics were comparable with those reported previously (Chen et al. 1999; Saleh et al. 1996, 1997b; Zidichouski et al. 1996) and synaptic responses mediated by CNQX-sensitive, non-N-methyl-D-aspartate (NMDA) receptors could be evoked in a majority of cells by stimulating afferent fibers near the ventral tip of the cerebellar peduncle (data not shown).

AVP depresses evoked EPSCs

In voltage-clamp mode, AVP depressed the evoked EPSC without changes in holding current. This effect was readily reversible on washout (Fig. 1A) and repeated application on the same cell did not produce desensitization. On cells that were treated with identical doses of AVP, the reductions in the EPSC were similar (1st dose 23.6 ± 6.8%, 2nd dose 24.8 ± 5.4%, n = 3, P > 0.05 paired t-test). The AVP-induced EPSC depression was dose dependent (5 μM, and there was no significant difference in the amount of depression at doses >1 μM (Fig. 1B). To differentiate where AVP acts to depress the EPSC, we employed the paired-pulse protocol where two identical afferent stimulations were given at a fixed 50-ms interval and a change in the ratio of the two responses was taken as an indicator for a presynaptic mechanism. AVP at a dose of 1 or 5 μM increased this ratio (Fig. 1C, averaged ratio for control: 1.25 ± 0.1, for AVP: 1.51 ± 0.08, n = 4, P < 0.05 paired t-test).

We further ruled out the postsynaptic involvement by examining the passive membrane conductances and glutamate receptor sensitivity on the postsynaptic cell. The AVP-induced depressant effect on the EPSC was not accompanied by postsynaptic changes in input resistance at holding potentials (−65 mV). Furthermore steady-state I-V curves generated by a slow ramp protocol (−120 to −30 mV) showed that AVP did
not change the \textit{I-V} relationship over a wide voltage range (Fig. 2A). The reduction in the EPSC did not appear to be due to an interaction with the postsynaptic glutamate receptors as inward currents induced by a brief bath application of AMPA (5 μM, 30 s) in control and in the presence of 1 μM AVP were also comparable (control: 61.5 ± 9.5 pA, in 1 μM AVP: 66 ± 9.6 pA, \( n = 4 \), \( P > 0.05 \), paired \( t \)-test, Fig. 2B). In the current-clamp mode, the action potentials evoked by depolarizing steps and the \textit{I-V} relationships generated by a series of current injections were not altered by AVP (Fig. 2, C and D). Collectively, these results suggest that AVP reduces the EPSC through a presynaptic action.

\textbf{AVP depresses the EPSC by acting at V\textsubscript{1} receptors}

As AVP receptors in the brain are mostly of V\textsubscript{1} type (Ostrowski et al. 1992; Tribollet et al. 1988), we tested if the observed EPSC effects were mediated by V\textsubscript{1} receptors using Manning compound, an AVP receptor blocker that is selective for the V\textsubscript{1} over the V\textsubscript{2} receptors. Cells were tested for AVP’s synaptic effects, and when they were recovered fully, Manning compound was perfused followed by AVP in the presence of AVP.

\textbf{FIG. 2.} \textit{A}: \textit{I-V} curves generated by steady-state slow ramp protocol from −120 to −30 mV over 20 s in control condition and in the presence of 1 μM AVP. \textit{B}: inward currents induced by a brief application of AMPA (5 μM, 30 s) in control condition and in the presence of AVP (1 μM, 3–4 min into treatment). \textit{C}: membrane potential changes to current steps. Note the resting membrane potential remains unchanged. \textit{D}: \textit{I-V} relationship derived from \textit{C}.

\textbf{FIG. 3.} \textit{V\textsubscript{1}-type receptors mediate AVP-induced synaptic effects.} \textit{A}: traces from a representative cell in response to AVP alone and Manning compound plus AVP. Letters indicate traces taken at times shown in normalized responses. \textit{B}: EPSC responses to drug treatments are normalized against their control values, which are at 100% (this and subsequent figures of EPSC responses). Normalized EPSC response to AVP (1 μM) and blockade by the V\textsubscript{1} receptor antagonist, Manning compound (MC, 10 μM). Data are means and SE (\( n = 5 \)).
Manning compound. AVP (1 μM) alone induced a reduction in the EPSC (18.7 ± 3.1%, n = 5), whereas the same dose produced no significant EPSC reduction in the presence of 10 μM Manning compound (6.9 ± 4.3%, n = 5, P < 0.05 vs. AVP alone, paired t-test, Fig. 3).

**Aminopeptidase inhibition induces V1-mediated synaptic effects**

AVP is cleaved by aminopeptidases and blockade of these aminopeptidases prevents AVP metabolism in vivo (Burback and Lebouille 1983; Stark et al. 1989). The PBN previously has been found to be rich in peptidases, and their inhibition greatly increases the efficacy of several of their peptide substrates in the region (Saleh et al. 1996). To test whether inhibition of the aminopeptidase that cleaves AVP has any effects on AVP-induced synaptic changes, cells were tested sequentially for AVP (1 μM) and amastatin [an inhibitor of aminopeptidase that cleaves AVP and oxytocin (Stancampiano et al. 1991), 10 μM] plus AVP at the same dose. AVP alone resulted in an EPSC reduction (17.4 ± 4.2%, n = 7). Amastatin also caused a reduction in the EPSC (17.7 ± 2.1%, n = 7) comparable with that induced by AVP at 1 μM, and additional AVP, in the presence of amastatin, further depressed the EPSC (28.6 ± 5.8%, n = 7, P < 0.05 vs. AVP alone, paired t-test, Fig. 4A).

Because amastatin prevents AVP degradation, we asked if the amastatin-induced change in the EPSC was a result of an action of endogenously released AVP in the PBN. Amastatin depressed the evoked EPSC on its own (16.7 ± 4.4%, n = 5), and this effect was blocked by 10 μM Manning compound (4.4 ± 5.1%, n = 5, P < 0.05 vs. amastatin alone, paired t-test, Fig. 4B). These results indicate that the action of endogenous AVP in the PBN is terminated by an amastatin-sensitive aminopeptidase.

**Lack of synergy between AVP and CRH**

It is well established that AVP and CRH have synergistic effects on the hypothalamic-pituitary-adrenal axis (Antoni 1993; Plotsky 1991) and both peptides are present in the PBN (Merchanthaler et al. 1982; Palkovits et al. 1985; van Zwieten et al. 1996). We therefore examined the effects of CRH on excitatory synaptic transmission and its interaction with AVP. Bath application of 1 μM CRH slightly, but significantly, increased the amplitude of the evoked EPSC (10.1 ± 6.8%, n = 6) without a change in I-V relationships generated by a ramp protocol (data not shown). AVP alone depressed the EPSC by 16.1 ± 4.5% (n = 6), and coapplication of AVP and CRH resulted in an attenuated action of AVP (7.1 ± 6.6%, n = 6, P < 0.05 vs. AVP alone, paired t-test; Fig. 5). There was therefore no synergy between these two peptides on the excitatory synaptic transmission in the PBN.
pyramidal cells in the hippocampus by exciting local GABAergic interneurons (Albeck et al. 1990; Smock et al. 1990). Although such a mechanism could account for our findings, we have never seen a postsynaptic effect of AVP on any neuron in the vicinity of the PBN which would be expected if it were to excite a GABAergic interneuron. Furthermore as AVP reduced the EPSC even though GABA_A receptors were blocked with picrotoxin, at least this subclass of receptors would not appear to have been involved. Although we cannot eliminate the participation of GABA_B receptors, in our previous work (Chen et al. 1999), the magnitude and onset of GABA_B receptor activation was very different from what we saw in the present experiments. We believe that the most likely explanation for our effects is that AVP was acting at presynaptic glutamatergic terminals to reduce glutamate release. Although the mechanism of the inhibition is not yet determined, a likely scenario would be inhibition of a calcium current in the presynaptic terminal such as has been suggested for a number of other presynaptic receptors (Colmers and Bleakman 1994; Shapiro and Hille 1993). Alternatively, increases in K^+ conductances in axon endings would reduce equally the likelihood of transmitter release, as is the case for opioid peptides (Simmons and Chavkin 1996; Vaughan et al. 1997).

Even at maximal doses, the effects of AVP on the evoked potentials seldom exceeded a 30% reduction. This contrasts with the action of some other peptides we have previously tested in the PBN such as substance P (Saleh et al. 1996) and cholecystokinin (Saleh et al. 1997b) where maximal doses of the peptides can suppress completely the EPSC. The much less dramatic action of AVP could be because of the fact that there are fewer receptors for this peptide, making it relatively ineffective in altering ionic conductances in the terminals; an alternate possibility is that only a very few terminals from a heterogeneous population are strongly affected by the peptide. We cannot distinguish between these possibilities as our afferent stimulation undoubtedly activates a nonhomogeneous population of afferents.

In this study, AVP’s synaptic action could be blocked by a V_1 receptor antagonist, which is consistent with V_1 receptors mediating the reduction in the EPSC. The Manning compound at the dose used in this study also blocks oxytocin receptors, and AVP has been shown previously to have activity at oxytocin receptors in the brain (Muhlethaler et al. 1983). Preliminary results from a cell that was responsive to AVP showed that oxytocin caused a reduction in the EPSC, therefore it is possible that some of the AVP-induced synaptic effects we observed could result from AVP acting at the oxytocin receptors. However, it must be noted that neither oxytocin receptors (Tribollet et al. 1989) nor oxytocin receptor mRNA have been localized in the PBN.

ChTX incubation, which blocks further activation of the Gs, did not alter AVP-induced EPSC depression, suggesting that Gs-coupled V_2 receptors are not involved. V_1 receptors are coupled to Gq and other G proteins to activate phospholipase C (Moriarty et al. 1989; Nabika et al. 1985; Naro et al. 1997). This coupling has been reported to be PTX sensitive or resistant depending on the expression system used (Lynch et al. 1986; Moriarty et al. 1989). It now is appreciated that there are numerous subtypes of G proteins that are differentially sensitive to PTX (Fields and Casey 1997). In particular, G_{sz}, which is coupled to N-type Ca^{2+} channels is PTX resistant (Jeong and Ikeda 1998). We found that incubation of slices with PTX

![FIG. 6. AVP acts through a cholina toxin (ChTX)- and pertussis toxin (PTX)-insensitive pathway. Slices were incubated for 20 h in 3 ml of artificial cerebrospinal fluid, 0.5 μg/ml PTX or 1 μg/ml ChTX before recording began. A: traces from representative cells showing AVP-induced EPSC responses in control and PTX- and ChTX-treated slices. B: averaged AVP-induced EPSC reduction in control and PTX- and ChTX-treated slices. Numbers are cells recorded in each condition. AVP depresses EPSC via a ChTX- and PTX-insensitive mechanism]

AVP receptor transduction is accomplished via G-protein-coupled second-messenger systems (Moriarty et al. 1989; Nabika et al. 1985). We therefore used ChTX and PTX to determine if there was G protein involved in the signaling process. Slices were incubated in control ACSF or one of the toxins in ACSF (ChTX: 1 μg/ml; PTX: 0.5 μg/ml) for ~20 h before we began recording. ChTX incubation irreversibly activates the Gs and therefore occludes the effects of an agent that also uses Gs coupling. PTX irreversibly disables the Gi protein, hence uncoupling the agonist from all the downstream effectors. AVP-induced EPSC depression was 26.2 ± 7.8% in control slices (n = 5), 16.5 ± 5.5% in PTX-treated slices (n = 5), and 30.4 ± 3.6% in ChTX-treated slices (n = 4, Fig. 6). One-way ANOVA revealed no significance among the three groups, rejecting the seemingly attenuated response to AVP in PTX-treated slices. There were no changes in the resting membrane potential (RMP) or input resistance (I_p) in control, ChTX- or PTX-treated slices (control: RMP = -62.7 ± 1.7 mV, I_p = 580 ± 89 μMΩ, n = 5; ChTX-treated: RMP = -63.2 ± 0.8 mV, I_p = 597 ± 51 μMΩ, n = 4, and PTX-treated: RMP = -63.4 ± 2.1 mV, I_p = 535 ± 89 μMΩ, n = 5, one-way ANOVA, P > 0.05).

**DISCUSSION**

The results presented in this work demonstrate that AVP depressed the evoked EPSC in the PBN, possibly through an action at V_1 AVP receptors on presynaptic terminals. Aminopeptidase inhibition caused a reduction in the EPSC that could be blocked by a V_1 receptor antagonist, suggesting the peptide is released (and degraded) in the PBN. AVP produced further EPSC depression in the presence of amastatin. AVP’s synaptic effects were reduced in the presence of CRH and were mediated by a ChTX- and PTX-insensitive signaling pathway.

**AVP depresses EPSC by acting on the V_1 receptors presynaptically.**

It is reported that AVP reduces the population spikes of the pyramidal cells in the hippocampus by exciting local GABAergic interneurons (Albeck et al. 1990; Smock et al. 1990). Although such a mechanism could account for our findings, we have never seen a postsynaptic effect of AVP on any neuron in the vicinity of the PBN which would be expected if it were to excite a GABAergic interneuron. Furthermore as AVP reduced the EPSC even though GABA_A receptors were blocked with picrotoxin, at least this subclass of receptors would not appear to have been involved. Although we cannot eliminate the participation of GABA_B receptors, in our previous work (Chen et al. 1999), the magnitude and onset of GABA_B receptor activation was very different from what we saw in the present experiments. We believe that the most likely explanation for our effects is that AVP was acting at presynaptic glutamatergic terminals to reduce glutamate release. Although the mechanism of the inhibition is not yet determined, a likely scenario would be inhibition of a calcium current in the presynaptic terminal such as has been suggested for a number of other presynaptic receptors (Colmers and Bleakman 1994; Shapiro and Hille 1993). Alternatively, increases in K^+ conductances in axon endings would reduce equally the likelihood of transmitter release, as is the case for opioid peptides (Simmons and Chavkin 1996; Vaughan et al. 1997).

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resulted in a smaller, but insignificant, AVP-induced reduction in EPSC. It should be noted that PTX treatment could differentially affect the pre- or postsynaptic elements (Colmers and Pittman 1989) and the mechanisms that mediate excitatory or inhibitory synaptic events (Thompson and Gahwiler 1992). PTX incubation does block pre- and postsynaptic effects of baclofen (Thompson and Gahwiler 1992), so it was taken as adequate PTX treatment when it blocked baclofen-induced hyperpolarization. The dose and duration of PTX incubation appeared adequate because parallel experiments showed that PTX treatment blocked baclofen-induced hyperpolarization of the PBN cells (Chen et al. 1999). This would at least suggest that the AVP receptors on the terminals are coupled to a G protein that is either wholly or partially resistant to PTX.

**Aminopeptidase inhibition enhances AVP’s synaptic effects**

Released AVP is terminated by aminopeptidase cleavage (Burback and Lebouille 1983; Stark et al. 1989). Inhibition of this group of aminopeptidase has been shown to increase AVP-induced synaptic responses in the hippocampus and the supraoptic nucleus (Kombian et al. 1997; Miura et al. 1997). There are abundant peptidases in the PBN, and their inhibition leads to a remarkable shift in the dose response of peptides. For example, phosphoramidon (an inhibitor of endopeptidases that degrade substance P) greatly enhance the effects of the peptide in the PBN (Saleh et al. 1996). This prompted us to explore whether AVP’s action in the PBN is modulated by ongoing degradation by peptidases. Pretreatment with amastatin, an inhibitor of the peptidase that cleaves AVP and oxytocin (Burback and Lebouille 1983; Stancampiano et al. 1991), caused a reduction in the EPSC, and this effect could be blocked by a V1 receptor antagonist. This suggests that the endogenously released AVP in the PBN is dynamically terminated by peptidases.

If endogenous AVP is present, one would expect that application of an antagonist would increase the size of the EPSC by blocking AVP’s depressant action. We saw no such effect, suggesting that there is efficient cleavage by the aminopeptidase so that the endogenous AVP does not reach a concentration high enough to reduce EPSC. This enzymatic activity could account for the relatively high threshold for the AVP-induced effects. Reduced responses at the highest dose we tested may be a result of either fast desensitization associated with high doses or an unknown mechanism whereby the activity of proteolytic enzymes is triggered. Because amastatin-induced EPSC reduction is a V1-receptor-mediated phenomenon, the fact that AVP and amastatin combined to produce EPSC reduction greater than the maximal AVP-induced depression supports our notion that AVP is actively removed in the PBN by aminopeptidase activity.

**AVP and CRH do not synergize to suppress EPSCs in the PBN**

CRH neurons in the PBN are found to regulate locus coeruleus cell activity and AVP release in the hypothalamus in the intact animal (Borsody and Weiss 1996; Carlson et al. 1994) and to excite dissociated or cultured neurons in vitro (Aldenhoff et al. 1983; Fox and Gruol 1993). Our results that CRH slightly increased the EPSC is consistent with its increasing Ca²⁺ entry and/or decreased K⁺ conductance (Aldenhoff et al. 1983; Fox and Gruol 1993; Lee and Tse 1997; Yu and Shinnick-Gallagher 1998). The marginal increase could be explained by the finding that CRH receptors in the brain are low in normal conditions and are inducible on increased demand (Mansi et al. 1996). It is also possible that there are subtle actions of CRH on membrane conductances that were not revealed in our analysis and could account for the small increase in the EPSC. Further studies will be required to investigate more fully the action of CRH in this nucleus.

In the parvocellular PVN, cells coexpressing CRH and AVP are capable of switching transmitter synthesis in favor of one over the other as demanded and the peptides have synergistic effects on the corticotropes (Antoni 1993; Leong 1988; Sawchenko 1987). Corticotropes primed by one of the peptides respond to the other with potentiation, possibly as a result of cross-talk between messenger molecules. Rather than a synergistic interaction, we found a functionally antagonistic interaction. As the mechanism of action of the CRH-induced increase in the EPSC has not been investigated in the present study, we are not able to determine the basis for this antagonistic interaction. It could relate to the different subtype of AVP receptors in the CNS and the pituitary or even to the possibility that the receptors may not be colocalized on the same cells.

**Physiological relevance**

The PBN is implicated in cardiovascular and neuroendocrine regulation. Injection of AVP directly into the PBN (Berecek et al. 1984) or stimulation of vasopressinergic cell bodies in the hypothalamus (Pittman and Bagdan 1992; Pittman and Franklin 1985) changes cardiac performance, implicating AVP as a one of the mediators in this modulation. The means by which this could occur may be an action of AVP on glutamate transmission in the PBN such as that we have demonstrated in the present report.

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