Regulation of vasopressin secretion by ET\textsubscript{A} and ET\textsubscript{B} receptors in compartmentalized rat hypothalamo-neurohypophysial explants

Noreen F. Rossi

Departments of Medicine and Physiology, Wayne State University School of Medicine, and John D. Dingell Veterans Affairs Medical Center, Detroit, Michigan 48201

Submitted 29 July 2003; accepted in final form 25 November 2003


First published December 9, 2003; 10.1152/ajpendo.00344.2003.—The endothelins (ET) have been implicated in vasopressin (AVP) release in vivo and in vitro. The effects of ET in this system are complex, and the net AVP secretory response likely depends on a unique combination of ET isoform, ET receptor subtype, and neural locus. The purpose of these studies was to examine the role of ET receptor subtypes at hypothalamic vs. neurohypophysial sites on somatodendritic and neurohypophysial AVP secretion. Experiments were done in cultured explants of the hypothalamo-neurohypophysial system of Long Evans rats. Either the whole explant (standard) or only the hypothalamus or posterior pituitary (compartmentalized) was exposed to log dose increases (0.01–10 nM) of the agonists ET-1 (ET\textsubscript{A} selective), ET-3 (nonselective), or IRL-1620 (ET\textsubscript{B} selective) with or without selective ET\textsubscript{A} (BQ-123, 2–200 nM) or ET\textsubscript{B} (IRL-1038, 6–600 nM) receptor antagonism. In standard explants, ET-1 and ET-3 dose-dependently increased, whereas IRL-1620 decreased net AVP release. Hypothalamic ET\textsubscript{B} receptor activation increased both somatodendritic and neurohypophysial AVP release. At least one intervening synapse was involved, as tetrodotoxin blocked the response. Activation of ET\textsubscript{A} receptors at the hypothalamic level inhibited, whereas ET\textsubscript{A} receptor activation at the posterior pituitary stimulated, neurohypophysial AVP secretion. Antagonism of hypothalamic ET\textsubscript{A} receptors potentiated the stimulatory effect of ET-1 and ET-3 on neurohypophysial secretion, an effect not observed with ET\textsubscript{B} receptor-induced somatodendritic release of AVP. Thus the response of whole explants reflects the net result of both stimulatory and inhibitory inputs. The integration of these excitatory and inhibitory inputs endows the vasopressinergic system with greater plasticity in its response to physiological and pathophysiological states.

ENDOTHELINS (ET) not only exert powerful direct vasoactive effects but can act as neurotransmitters within the central nervous system (4, 10, 15). Specifically, ET peptides have been implicated in the regulation of vasopressin (AVP) secretion in vivo (20, 23) and in vitro (19–22, 24, 26, 29). The neural loci involved in AVP release contain both ET-1 and ET-3 isoforms, their respective mRNAs, and ET-converting enzyme activity (13, 16, 28, 31). ET\textsubscript{A} and ET\textsubscript{B} receptors exist in roughly equal numbers within the hypothalamo-neurohypophysial system. Importantly, ET\textsubscript{A} receptors are located on magnocellular neurons within the supraoptic and paraventricular nuclei as well as nonvasopressinergic neurons within the hypothalamus (12, 30). ET\textsubscript{B} receptors are distributed in the hypothalamus, most prominently in the organum vasculosum of the lamina terminais and median eminence (30). Moreover, ET colocalizes with AVP in secretory vesicles of the neural lobe and exerts actions on the posterior pituitary itself (16, 21, 31). These sites are all integrally involved in the control of AVP secretion.

The effects of ET in this system are complex, depending on the isoform, the locus of action, and the receptor subtype involved, and remain to be characterized fully. The ET\textsubscript{A} receptor has higher affinity for ET-1, whereas the ET\textsubscript{B} receptor has roughly equal affinities for ET-1 and ET-3 (5). After the initial report that ET induced AVP secretion by rat hypothalamus in vitro (26), it was shown in compartmentalized hypothalamo-neurohypophysial explants that a nanomolar concentration of ET-3 stimulated AVP release at the posterior pituitary rather than at a hypothalamic site (21). Electrophysiological studies demonstrated that ET-1 inhibits phasically firing magnocellular neurons (a characteristic of AVP rather than oxytocin cells) within the supraoptic nuclei but exerts excitatory actions on neurons within the anteroventral third ventricular (Av3V) region (29). Neurons within the Av3V area project to the supraoptic nuclei. Thus it is likely that at least one synapse intervenes between the neurons stimulated by ET-1 in the Av3V region and the vasopressinergic neurons.

We hypothesize that release of AVP by the neurohypophysis will be increased by hypothalamic ET\textsubscript{B} receptor activation via a mechanism that involves at least one intervening synapse and will be directly stimulated by ET\textsubscript{A} receptor activation at the posterior pituitary. We further propose that somatodendritic AVP secretion at the hypothalamic level will differ from that observed from the neural lobe in response to the distinct subtype of ET receptor activated. The present studies were carried out in compartmentalized hypothalamo-neurohypophysial explants, thereby permitting evaluation of AVP release at both sites independent of potential systemic influences, such as baroreflex modulation (4, 23) or ET-induced actions on cerebral microvasculature (25).

METHODS

Experiments were performed on 125- to 150-g male Long-Evans rats. They were housed at constant temperature on a 12:12-h light-dark cycle and given free access to water and rodent chow. All procedures were reviewed and approved by the institutional Animal Investigation Committee and were in compliance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health.

Dissection and Culture of Explants

Explants of the hypothalamo-neurohypophysial complex (HNC) were dissected as described earlier (27). The explants contained both

Address for reprint requests and other correspondence: N. F. Rossi, Depts. of Medicine and Physiology, Wayne State Univ. School of Medicine, 4160 John R #908, Detroit, MI 48201 (E-mail: nrossi@med.wayne.edu).

http://www.ajpendo.org
supraoptic nuclei with intact axonal projections to the neural lobe with the intermediate lobe attached. The organum vasculosum of the lamina terminalis and the arcuate, suprachiasmatic, preoptic, and ventromedial nuclei were present. The subforminal organ and paraventricular nuclei are not included.

**Explants in standard culture.** Explants cultured by the standard method were placed ventral side down onto Teflon mesh into separate incubation wells containing culture medium composed of Ham’s F-12 nutrient mixture supplemented with 5.5 mM dextrose, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Grand Island, NY) fortified with 20% fetal bovine serum (HyClone, Logan, UT) with a final osmolality of 297 ± 1 mosmol/kgH2O. Medium was changed every 24 h after an experimental protocol. Both hypothalamic (HT) and posterior pituitary (PP) components were in the one culture well and exposed to the same medium.

**Explants in compartmentalized cultures.** After dissection, compartmentalized explants were immediately placed into custom-fabricated sterile incubation chambers that have a two-piece barrier separating the hypothalamus from the posterior pituitary (7, 21, 22). The explants were positioned onto Teflon mesh with the intact infundibular stalk lying in a 0.33-mm-wide by 0.2-mm-deep notch in the lower half of the barrier. The upper half of the barrier was then slid into place. The barrier interfaces and gaps were sealed with sterile silicone grease to prevent peptide degradation (27). Each compartment of the chamber was filled with culture medium. The medium in each compartment was changed every 24 h. When properly positioned and sealed, the only communication between the two compartments was neuronal. Diffusion via the infundibular recess could not be entirely eliminated; however, the extent of leaking between compartments was assessed at the end of the experiment for each explant by adding 3H-labeled water (as a 150 mM NaCl solution) to one compartment and measuring its radioactivity in the opposite compartment. Explants displaying >0.05%/h leak were discarded from analysis. At the end of the experiments, the upper barrier was removed, and each explant was scrutinized under a stereomicroscope (magnification ×25) for trauma to the infundibular stalk. Explants were classified as damaged if there was visual evidence of attenuation or lengthening of the stalk (commonly seen with excessive traction), nicks or cuts in the fibers of the stalk or any part of the explant (usually incurred during positioning of the explant within the chamber), and complete separation of the hypothalamus and pituitary. Tissues exhibiting damage to the stalk were discarded prospectively from further analysis. All explants were kept at 37°C under a humidified atmosphere of 95% O2-5% CO2 at pH 7.4.

**Sampling Procedures**

All protocols were performed with 0.05 mg/ml bacitracin (Sigma, St. Louis, MO) to prevent peptide degradation (27). Each compartment was changed every 24 h. When properly positioned and sealed, the only communication between the two compartments was neuronal. Diffusion via the infundibular recess could not be entirely eliminated; however, the extent of leaking between compartments was assessed at the end of the experiment for each explant by adding 3H-labeled water (as a 150 mM NaCl solution) to one compartment and measuring its radioactivity in the opposite compartment. Explants displaying >0.05%/h leak were discarded from analysis. At the end of the experiments, the upper barrier was removed, and each explant was scrutinized under a stereomicroscope (magnification ×25) for trauma to the infundibular stalk. Explants were classified as damaged if there was visual evidence of attenuation or lengthening of the stalk (commonly seen with excessive traction), nicks or cuts in the fibers of the stalk or any part of the explant (usually incurred during positioning of the explant within the chamber), and complete separation of the hypothalamus and pituitary. Tissues exhibiting damage to the stalk were discarded prospectively from further analysis. All explants were kept at 37°C under a humidified atmosphere of 95% O2-5% CO2 at pH 7.4.

**Analytical Methods**

AVP content of the medium was measured by radioimmunoassay with methods previously reported from our laboratory (20–24). Samples of medium were diluted 1:50 and directly assayed in duplicate. Media from one set of experiments were assayed together to avoid interassay variability. Standards were prepared with purified AVP (Ferring, Malmo, Sweden). [125I]iodotyrosyl-AVP was used as the tracer (Amersham Pharmacia Biotech, Piscataway, NJ). Anti-AVP serum no. 2849 (prepared by J. Durr, Veterans Affairs Medical Center, Tampa, FL) was used at a final dilution of 1:3.6 × 105.

Medium osmolality was measured by freezing point depression (Precision Systems, Sudbury, MA).

**Statistical Analysis**

Release rates for basal and test periods were calculated and corrected for volume and degradation as reported previously (27). All comparisons of AVP release between basal period and test period release rates were performed on the absolute release rates after correction for degradation. Each explant acted as its own control.
Because basal secretory rates varied from explant to explant, the release of AVP during the test hour was normalized as the percentage of AVP released during the preceding basal hour for the same compartment. Comparisons among test periods from different groups of explants were then performed on the normalized release rates.

Differences between basal and test period release of AVP in the same explants were compared using the paired t-test. Comparisons among test periods from separate groups of explants were assessed by analysis of variance and the Tukey-Kramer test for multiple comparisons. All data are expressed as means ± SE. P values < 0.05 were considered significant.

RESULTS

Figure 1 shows the dose response for AVP release in response to the ET agonists by explants in standard culture. Concentrations of ET-1 and ET-3 at the higher range significantly stimulated AVP secretion, whereas release declined at higher doses of IRL-1620. Average overall basal AVP secretion was 74 ± 24 pg-HNC⁻¹·h⁻¹ (n = 91). Degradation averaged 3.2 ± 0.7% and did not exceed 6.8%. Time control experiments with medium added during both basal and test periods showed no change in the AVP secretory rate: 100 ± 30 vs. 93 ± 25%·HNC⁻¹·h⁻¹ (n = 6).

BQ-123 dose-dependently inhibited the stimulatory effect of 1 nM ET-1 or ET-3 but did not change AVP release by 0.01 nM IRL-1620 (Fig. 2). Average basal AVP secretion was 69 ± 22 pg-HNC⁻¹·h⁻¹ (n = 85). IRL-1038 significantly antagonized AVP release to IRL-1620 but did not inhibit the response to ET-1 (Fig. 3). Notably, the ETB blocker did not block the effect of the nonselective agonist ET-3, but at the highest dose IRL-1038 significantly potentiated the AVP secretory rate to 1 nM ET-3. Basal AVP secretion for this group of experiments averaged 65 ± 21 pg-HNC⁻¹·h⁻¹ (n = 74). When administered alone, neither BQ-123 nor IRL-1038 at the highest doses tested changed AVP release: 100 ± 20 (basal) vs. 103 ± 40%·HNC⁻¹·h⁻¹ (200 nM BQ-123, n = 6); 100 ± 28 (basal) vs. 108 ± 31%·HNC⁻¹·h⁻¹ (600 nM IRL-1038, n = 7).

When medium was added to both sides of the barrier in compartmentalized explants during both basal and test periods, AVP secretory rates were unchanged from hypothalamus (100 ± 27 vs. 99 ± 40%·HT⁻¹·h⁻¹, n = 4) and from posterior pituitary (100 ± 31 vs. 66 ± 39%·PP⁻¹·h⁻¹, n = 4). Figure 4 shows the AVP release when the ET agonists were added to the hypothalamic compartment. Figure 4A depicts release of AVP by the hypothalamus, and Fig. 4B shows secretion by the posterior pituitary during the test period. Basal AVP release averaged 23 ± 5 pg·HT⁻¹·h⁻¹ by the hypothalamus and 37 ± 6 pg·PP⁻¹·h⁻¹ by the pituitary (n = 71). Maximal stimulation with IRL-1620 was observed at a concentration of 0.01 nM IRL-1620 (414 ± 89%·PP⁻¹·h⁻¹, P < 0.01 vs. basal, n = 6).

Figure 5 shows the effect of BQ-123 or IRL-1038 on AVP secretion by the hypothalamus and the neurohypophysis when the ET agonists and antagonists are applied to the hypothalamic side. Hypothalamic AVP release induced by the nonselective ETB agonist ET-3 was significantly lower in the presence of IRL-1038. Although pituitary release of AVP in these explants was lower (53 ± 20%·basal·PP⁻¹·h⁻¹) when ET-3 was added in the presence of IRL-1038 than with ET-3 alone (81 ± 43%·basal·PP⁻¹·h⁻¹), this did not achieve statistical significance. This is due, in part, to the difficulty in demonstrating inhibition when the release rate was already very low. ETB antagonism with BQ-123 potentiated AVP release by the neural lobe in response to ET-1 or ET-3 but did not alter AVP released into the hypothalamic compartment. Pituitary AVP

Fig. 1. Vasopressin (AVP) release during the test period in response to endothelin (ET)-1, ET-3, and IRL-1620 in explants of hypothalamo-neurohypophysial complex (HNC) cultured by the standard methods (basal release not shown). Each point represents 1 set of explants. Values are means ± SE; n = 6–8 for each observation. *P < 0.05 vs. basal at each dose of ET-1; †P < 0.05 vs. basal for 1 and 10 nM ET-3, and ‡P < 0.05 vs. basal for 0.01 and 0.1 nM IRL-1620.

Fig. 2. Effect of ETₐ antagonism by BQ-123 on AVP release by 1 nM ET-1, 1 nM ET-3, and 0.01 nM IRL-1620 in HNC explants cultured by the standard methods. Values are means ± SE; n = 6–8 for each observation. *P < 0.025 vs. basal (not shown; see results); †P < 0.05 vs. agonist alone.

Fig. 3. Effect of ETₐ antagonism by IRL-1038 on AVP release by 1 nM ET-1, 1 nM ET-3, and 0.01 nM IRL-1620 in HNC explants cultured by the standard methods. Values are means ± SE; n = 6–8 for each observation. *P < 0.05 vs. basal (not shown; see results); †P < 0.05 vs. agonist alone.
release in response to 1 nM IRL-1620 was also higher in the presence of the ET\(\text{A}\) inhibitor. Significantly, BQ-123 reversed the decline in hormone release with the highest dose of IRL-1620 tested: 93 ± 18\%\,PP\,\text{h}^{-1} (10 nM IRL-1620, \(n = 10\)) vs. 340 ± 75\%\,PP\,\text{h}^{-1} (10 nM IRL-1620 plus 20 nM BQ-123, \(n = 6\), \(P < 0.05\) vs. IRL-1620 alone); but it had no effect on pituitary AVP release to 0.01 nM IRL-1620 (323 ± 54\%\,PP\,\text{h}^{-1}, \(n = 5\)). In contrast, IRL-1038 blocked the maximal stimulation by 0.01 nM IRL-1620 (88 ± 54\%\,PP\,\text{h}^{-1}, \(n = 6\), \(P < 0.025\)). Overall, absolute basal AVP release in this group was 21 ± 8 pg\,\text{PP}\,\text{h}^{-1}\,\text{HT}\,\text{h}^{-1} by the hypothalamus and 34 ± 8 pg\,\text{PP}\,\text{h}^{-1}\,\text{PP}\,\text{h}^{-1} by the posterior pituitary (\(n = 66\)).

Likewise, Fig. 6 illustrates the AVP secretory response by the neurohypophysis when the ET agonists were added to the posterior pituitary side of the barrier. In these explants, the average basal AVP secretory rate was 39 ± 12 pg\,\text{PP}\,\text{h}^{-1}\,\text{h}^{-1} (\(n = 52\)). The effects of ET agonists and antagonists added to the posterior pituitary on AVP secretion are displayed in Fig. 7. Average basal neural lobe AVP release was 34 ± 8 pg\,\text{PP}\,\text{h}^{-1}\,\text{h}^{-1} in this group (\(n = 53\)).

The effects of tetrodotoxin on AVP release into the pituitary compartment by stimulation of the pituitary with ET-1 or the hypothalamus with IRL-1620 are depicted in Table 1. When IRL-1620 was added together with tetrodotoxin to the hypo-
ET-INDUCED AVP RELEASE IN COMPARTMENTALIZED EXPLANTS

The present studies highlight the complex interactions that occur when multiple isoforms of a peptide, such as ET, act via distinct receptor subtypes located on different neuronal elements of the same or synapticlly related neurons. The hypothalmo-neurohypophysial explant in standard culture provides a useful experimental model for the study of AVP release. Even so, these explants possess more than one site for ET peptide action. The AVP released into the medium will be the net hormone secreted in response to both inhibitory and excitatory inputs from both somatodendritic and neural lobe (axonal) elements. Our data with explants in such standard cultures confirmed earlier results showing a progressive net increase in AVP release with higher concentrations of ET-1 by perfused hypothalami (26) or ET-3 by hypothalmo-neurohypophysial explants in standard culture (21). Because ET-3, which has roughly equal affinities for ETA and ETB receptors (5), potently stimulates AVP secretion from the neural lobe (26) and neurons in the Av3V region that possess mostly ETB receptors were excited by ET-1 (29), the dose-dependent decrease in AVP secretion with the selective ETB agonist was somewhat unexpected. However, this finding was further corroborated by the observation that, once the ETB receptors were maximally blocked with IRL-1038, then unopposed ETA agonism resulted in potentiation of the neurohormone secretory response by ET-3. Overall, these results supported a stimulatory role for ETA receptor activation on AVP release. That there are at least two sites of stimulatory action, one via ETA receptors and another via ETB receptors within the structures included in the explants is suggested by the observation that picomolar concentrations of the ETB agonist also stimulated AVP and could be selectively and dose-dependently inhibited by IRL-1038. In addition, any working model should be able to reconcile the secretory data with electrophysiological studies that have shown that ET-1 inhibits phasically firing magnocellular neurons (29) whose somata are primarily endowed with ETA receptors (30) but excites neurons in the Av3V region (29), which encompasses the organum vasculosum of the lamina terminalis where ETB receptors are located (30).

The experiments using compartmentalized hypothalmo-neurohypophysial explants provide new insights into the interplay of ET receptor subtypes and sites of action that result in AVP secretion. Application of the lowest concentration of IRL-1620 exclusively to the hypothalamus clearly resulted in AVP release by the neural lobe. The lack of stimulation by 10 nM IRL-1620 suggests that, at higher doses the ETB agonist begins to bind to inhibitory hypothalamic ETA receptors similar to binding characteristics of ET ligands reported in rat anterior pituitary (8). This interpretation is also consistent with

Table 1. AVP release by posterior pituitary in the presence and absence of TTX

<table>
<thead>
<tr>
<th>Condition</th>
<th>Basal Release, pg PP⁻¹ h⁻¹</th>
<th>Test Release, pg PP⁻¹ h⁻¹</th>
<th>Normalized Basal Release, % basal PP⁻¹ h⁻¹</th>
<th>Normalized Test Release, % basal PP⁻¹ h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamic application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 nM IRL-1620</td>
<td>40±14</td>
<td>162±22*</td>
<td>100±35</td>
<td>405±55†</td>
</tr>
<tr>
<td>0.1 nM IRL-1620 + 10 μM TTX</td>
<td>19±4</td>
<td>25±9†</td>
<td>100±21</td>
<td>132±47§</td>
</tr>
<tr>
<td>Putitious application</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 nM ET-1</td>
<td>57±17</td>
<td>313±89*</td>
<td>100±30</td>
<td>549±156†</td>
</tr>
<tr>
<td>10 nM ET-1 + 10 μM TTX</td>
<td>28±11</td>
<td>152±46*</td>
<td>100±39</td>
<td>543±164†</td>
</tr>
</tbody>
</table>

Values are means ± SE. PP, posterior pituitary; TTX, tetrodotoxin; ET-1, endothelin 1. Standard medium was added to opposite compartment. *P < 0.05 vs. basal release; †P < 0.05 vs. normalized basal release; §P < 0.025 vs. test release to IRL-1620; ¥P < 0.025 vs. normalized test release to IRL-1620.
electrophysiological findings that ET-1, an ET<sub>A</sub> agonist, inhibits presumptive vasopressinergic neurons within the supraoptic nucleus (29) and by the present observation that concurrent blockade of hypothalamic ET<sub>A</sub> receptors with BQ-123 resulted in significant neurohypophysial AVP secretion by ET-1 or ET-3.

Tetrodotoxin blockade of the hypothalamic IRL-1620-induced AVP release by the posterior pituitary further indicates that the stimulatory effect is not located on the magnocellular neuron itself but resides on neurons that project to the AVP secretory cell. Previous studies from our laboratory (22) implicate an N-methyl-D-aspartate receptor, but other receptors may be involved as well. Although the present data do not directly address the locus of these ET-responsive neurons or whether the projection is monosynaptic or polysynaptic, the Av3V region, which contains neurons excited by ET peptides (29) and includes the organum vasculosum of the lamina terminalis richly endowed with ET<sub>B</sub> receptors (30), is a candidate locus (Fig. 8).

That tetrodotoxin applied with ET-1 to the posterior pituitary did not block AVP release is not surprising. Nevertheless, receptor-binding studies in this brain region have not discriminated definitively between binding at the neural lobe and binding at the intermediate lobe of the posterior pituitary (12). The present data show that ET-1 indeed acts on receptors located directly on nerve terminals within the neural lobe rather than on the intermediate lobe, which is also present in the explant preparation (27).

The importance of somatodendritic release of AVP in autonomic control of magnocellular neurons and optimizing the efficiency of hormone release has become increasingly apparent (1, 3, 6, 11). AVP secreted into the hypothalamic compartment largely reflects somatodendritic release, although hormone release from axon projections that terminate in the proximate median eminence may also contribute. The pattern of AVP release into this compartment differed from simultaneously assessed neural lobe secretion. Selective ET<sub>A</sub> agonism failed to elicit AVP release into either compartment. In contrast, however, higher doses of ET-3 resulted in progressively greater AVP release by the hypothalamic components as did IRL-1620. This stimulatory effect was due to ET<sub>B</sub> receptor activation and involved at least one synapse. However, blockade of ET<sub>A</sub> receptors did not alter somatodendritic release as it did axonal AVP secretion into the pituitary compartment. Taken together with the observation that the mixed agonist increased hypothalamic AVP secretion, it appears that stimulation of the ET<sub>A</sub> receptor can inhibit the vasopressinergic neurons in the hypothalamus and modulate neurohypophysial AVP release without altering ET<sub>B</sub> receptor-induced somatodendritic secretion. Independent modulation of the vasopressinergic neuron itself by ET<sub>A</sub> receptors would permit even more plasticity in the net response to ET peptides.

Immunocytochemical studies have shown that AVP receptors exist on neurons within the supraoptic nucleus (2). Either direct AVP modulation of the AVP neuron (1, 11) or indirect action by depolarization of neighboring interneurons (3) can influence the firing pattern of the vasopressinergic cell so as to optimize AVP secretory activity (6). Therefore, under pathophysiological conditions such as septic or hemorrhagic shock, where circulating and/or brain ET levels are high (9, 14, 17, 18), activation of hypothalamic ET<sub>B</sub> receptors would result in a coordinated signal to the AVP neuron by somatodendritic release of AVP that would amplify hormone secretion by the neural lobe and result in sustained circulating levels of AVP.

Besides hypothalamic actions, ET peptides also exerted a stimulatory effect on the posterior pituitary. Specifically, ET-1 dose-dependently increased AVP release, which was inhibited by ET<sub>A</sub> antagonism. Analogous to the hypothalamus, ET<sub>B</sub> blockade prevented stimulation by the nonselective agonist ET-3 and uncovered the stimulatory effect of IRL-1620, presumably by displacing the ET<sub>B</sub> agonist to ET<sub>A</sub> sites. A direct inhibitory action was not definitively evident but may be suggested by the decrease in AVP secretion with the highest dose of ET-3. These findings are noteworthy in view of the colocalization of ET peptides and AVP in neurosecretory vesicles within the axon terminals in the neural lobe (16, 31). This would permit coreleased ET, yet another site for amplification (or modulation) of AVP secretion. Moreover, both the Av3V region and the posterior pituitary are devoid of the blood-brain barrier and may be responsive to circulating ET peptides as well as ET generated within the central nervous system.

In summary, the response of whole explants exposed to ET agonists and antagonists reflects the net result of both stimulatory and inhibitory inputs. Accordingly, even within the relatively simplified slice model of the whole hypothalmo-neurohypophysial explant, ET may exert complex actions on AVP release depending on the isoform, receptor subtype, and locus of the action. The integration of these excitatory and inhibitory inputs endows the vasopressinergic system with a greater plasticity in its response to physiological and pathophysiological states. In this context, the compartmentalized paradigm provides an approach for systematic analysis of these

**Fig. 8.** Putative schema of ET<sub>A</sub> and ET<sub>B</sub> receptor mechanisms involved in AVP secretion by a magnocellular neuron whose cell body is in the supraoptic nucleus and whose axon terminates in the PP. See CONCLUSION for complete explanation. Av3V, anteroventral 3rd ventricle region; ET<sub>A</sub>R, ET<sub>A</sub> receptor; ET<sub>B</sub>R, ET<sub>B</sub> receptor; (−) inhibitory; (+) excitatory.
influences on AVP secretion from both somatodendritic as well as neural lobe sources.

ACKNOWLEDGMENTS

We appreciate the technical assistance of Haiping Chen in performing the assays.

GRANTS

This work was supported by a Merit Award by the Department of Veterans Affairs to N. F. Rossi.

REFERENCES


