Aminopropionic Acid Receptors in Paraventricular Nucleus Mediate Pressor and Vasopressin Responses to Endothelin-1 in Subfornical Organ

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Endothelin-1 (ET-1) acts at selected brain loci to elicit a pressor response and secretion of vasopressin (AVP). Glutamatergic receptors of the *N*-methyl-D-aspartate (NMDA) subtype mediate ET-1–induced AVP secretion *in vitro,* but the role of glutamatergic receptors in the pressor response and the secretion of AVP *in vivo* has not been studied. We hypothesized that both the pressor response and AVP secretion in response to ET-1 microinjection into subfornical organ (SFO) would be suppressed by ionotropic glutamatergic receptor antagonists in the paraventricular nucleus (PVN). Sinoaortic denervated male Long Evans rats were equipped with intracerebral cannulae directed into the SFO and the magnocellular region of the PVN bilaterally. Experiments were performed 5 days later in conscious rats. Direct injection of 5 pmol ET-1 into the SFO resulted in a 20 ± 3 mm Hg increase in mean arterial pressure (MAP) (±SE) and a 14.1 ± 0.3 pg/ml increase in the mean plasma AVP level (±SE) ($P < 0.001$ vs. artificial CSF) that was blocked by selective ET_A inhibition. Neither the pressor response nor the increase in plasma AVP in response to ET-1 was altered despite prior injection of the NMDA blocker diclozipine (5 μg, MK801) into PVN bilaterally. In contrast, bilateral PVN injection with 6-cyano-7-nitroquinoxaline-2,3-dione (40 nmol, CNQX) prevented the pressor response (MAP ± SE, –4 ± 4 mm Hg) and also inhibited AVP secretion (mean AVP level ± SE, 0.16 ± 0.50 pg/ml) ($P < 0.001$ vs. vehicle in PVN after injection of ET-1 into SFO). These findings support the conclusion that both the pressor response and AVP secretion in response to ET-1 acting at the SFO are mediated by a non-NMDA, most likely an aminopropionic acid glutamatergic receptor within the PVN. Exp Biol Med 231:1075–1080, 2006

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Introduction

In addition to potent direct vasoactive effects, the endothelins (ETs) also act as neuromodulators within the central nervous system (1–4). All components of the ET system, including ET mRNA and peptides, ET receptors, and ET-converting enzyme activity, exist in neuroastroglial tissues (5, 6), particularly within loci implicated in the central regulation of cardiovascular function and vasopressin (AVP) secretion (6).

ET-1 injected into the lateral cerebral ventricles evokes a pressor response in rats (7–11) that is mediated by increased sympathetic efferent activity (8, 12, 13). In addition, ET-1 stimulates AVP secretion both *in vivo* (8, 9, 11, 14, 15) and *in vitro* (15–17). Early reports suggested that the pressor response is mediated by AVP secretion (7, 11). More-recent studies in the Brattleboro rat, a mutant strain lacking central AVP, indicate that the pressor response is independent of AVP (8).

Among the circumventricular organs, the subfornical organ (SFO) is generously endowed with ET receptors (18). Neural pathways project from SFO to supraoptic and paraventricular nuclei (PVN) (19, 20). ET-1 acting at the subfornical organ elicits an excitatory effect on magnocellular neurons within the PVN (1, 2). Notably, electrolytic lesions of the region anteroventral to the third ventricle interrupt projections from the SFO to the supraoptic nuclei and prevent AVP release but do not alter the pressor effect (9). In contrast, bilateral lesions of the PVN disrupt pathways from the anterior hypothalamus, including SFO, to nuclei in the medulla and spinal cord that mediate sympathetic outflow and cardiovascular responses, including the pressor effect of ET-1 (10). Bilateral PVN lesions also completely prevent the increase in plasma AVP levels

(10). These observations suggest that inputs from the anterior hypothalamus terminating on magnocellular neurons within the PVN and/or fibers of passage through the PVN to the supraoptic nuclei are necessary for AVP release.

Glutamate is a major excitatory neurotransmitter in the hypothalamus and interacts with two classes of inotropic receptors, *N*-methyl-D-aspartate (NMDA) and non-NMDA (21, 22). The non-NMDA receptors are subclassified into two groups, the kainic acid receptors and the amino-propionic acid (AMPA) receptors (23). Magnocellular neurons are richly endowed with glutamatergic receptors of both the NMDA and non-NMDA classes (21–24). Excitatory glutamatergic signals are among the major neural signals to the somata and dendrites of magnocellular neurons within the PVN (21, 24). Ionotropic glutamatergic receptors play a major role in modulating AVP release to osmotic stimuli (25, 26), and we have shown that NMDA receptors mediate ET_B receptor–induced AVP release *in vitro* (16). Electrophysiological data support a role for both NMDA and non-NMDA receptors in the activation of vasopressinergic neurons (22, 27, 28). Furthermore, intracerebroventricular injection of an AMPA receptor antagonist prevented the antidiuretic response, which is consistent with suppression of AVP release (29).

Glutamate has been implicated as the neurotransmitter that mediates the signals from neural fibers projecting from SFO to the PVN (2, 3). ET evokes efflux of glutamate from cultured rat astrocytes (31). Because bilateral lesions of the PVN abolish the pressor and AVP secretory response to ET-1, the present experiments were designed to test the hypothesis that NMDA and/or non-NMDA receptors within the PVN will mediate the pressor and AVP secretory response of ET-1 injected into the SFO.

Materials and Methods

Experiments were performed on 250–275-g male Long Evans rats (Harlan, Indianapolis, IN) housed at constant temperature with a 12:12-hr alternating light:dark cycle. Rats had unrestricted 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.

Surgical Procedures. Rats were anesthetized with sodium pentobarbital (40 mg/kg body wt ip). On day 1, all rats underwent sinoaortic denervation, performed as described previously (8, 9, 31), to prevent baroreflex inhibition of AVP secretion by the concurrent increase in arterial pressure associated with ET-1 injection (8). Catheters were inserted into the carotid artery and jugular vein, secured, tunneled subcutaneously, and exteriorized at the back of the neck (8). Catheter patency was maintained by filling the lumen with 50 μl sodium heparin (1000 U/ml). Guide cannulas (Plastics One, Inc., Roanoke, VA) were placed into the PVN bilaterally and SFO such that the tip of the infusion cannula corresponded to the stereotaxic coordinates for PVN and SFO (PVN: –1.5 anteroposterior, –0.6 and +0.6 mediolateral, and +8.6 dorsoventral; SFO: +0.1 lateral to midline, –0.9 caudal to bregma, and –5.8 ventral to skull surface). A dummy cannula was placed into the guide cannula to maintain patency until the day of testing. The rat was then allowed to recover.

Three days after surgery, each rat was tested while conscious for completeness of sinoaortic denervation. Only rats displaying >95% blunting of baroreceptor reflex changes in heart rate were used in the subsequent protocols.

Protocols. Rats were acclimated to the study chamber for 2 hrs daily for 4 days and were studied on day 5. Heart rate and arterial pressure were monitored continuously. Two series of experiments were performed.

Series 1. After a 30-min baseline period, 800 μl of arterial blood was obtained for measurement of plasma AVP, and the rat was transfused with an equal volume of blood as previously described (8, 9). The SFO was then injected with 250 nl of artificial cerebrospinal fluid (CSF), 5 pmol ET-1, 40 nmol BQ123 (ET_A antagonist), or ET-1 plus BQ123. A second blood sample was obtained 10 mins after SFO injection, the time of peak pressor response reported in previous experiments (15) and confirmed here.

Series 2. Baseline hemodynamic data and blood samples were obtained as in series 1. The PVN were then injected bilaterally with 250 nl of either vehicle (saline), 15 nmol dizocilpine maleate (MK801, NMDA antagonist), or 40 nmol 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, AMPA antagonist). Heart rate and arterial pressure were allowed to stabilize. Fifteen minutes later, the SFO was injected with artificial CSF, 5 pmol ET-1, or 40 nmol BQ123. Blood for plasma AVP was obtained 10 mins later. After completion of all protocols, the rats were euthanized with sodium pentobarbital (120 mg/kg body wt iv), and injection sites were verified histologically. Data from rats whose injections were not in the SFO and PVN were prospectively discarded from analysis. All chemicals were obtained from Sigma (St. Louis, MO).

Statistical Analysis. The Hemodynamic Monitoring Package (Biotech Products, Greenwood, IL) calculated the MAP and displayed the heart rate beat-by-beat for each experiment. Data were recorded continuously at 6 Hz, using a DAP 3216a/415 data-acquisition processor as the hardware platform. Mean arterial pressure (MAP) and heart rate were calculated as the running average of the final 3 mins of the period before the first blood sample was obtained (baseline) and the 7–10-min interval after SFO, just before the second blood sample was obtained. Plasma osmolality was measured by freezing-point depression, and the rest of the sample was stored at –70°C until radioimmunoassay, as previously described (15). Comparisons of MAP, heart rate, plasma osmolality, and plasma AVP concentration among the groups between baseline and the test periods were made using one-way analysis of variance (ANOVA). The Tukey Kramer *post hoc* test was performed to account for the

Table 1. Pressor and AVP Secretory Response 10 Minutes After SFO Injection[a]

SFO injection	No. of rats	Baseline MAP (mm Hg)	10-Min MAP (mm Hg)	ΔMAP (mm Hg)	Baseline AVP (pg/ml)	10-Min AVP (pg/ml)	ΔAVP (pg/ml)
Artificial CSF	10	122 ± 5	125 ± 2	3 ± 2	1.8 ± 0.6	2.1 ± 0.9	−0.3 ± 0.3
ET-1, 5 pmol	10	120 ± 4	141 ± 3*,***	20 ± 3*	0.8 ± 0.2	14.9 ± 0.3*,***	14.1 ± 0.3*
BQ123, 40 nmol	5	113 ± 3	117 ± 4	4 ± 3	1.3 ± 0.2	0.9 ± 0.3	−0.4 ± 0.4
ET-1 + BQ123	8	118 ± 6	122 ± 2**	4 ± 4**	1.1 ± 0.4	1.2 ± 0.4**	−0.1 ± 0.1**

[a] Values are mean ± SE.
*$P < 0.001$ vs. artificial CSF.
**$P < 0.001$ vs. ET-1.
***$P < 0.001$ vs. baseline.

problem of multiple comparisons. Differences during the test period among groups were analyzed by two-way ANOVA. All data are reported as mean ± SE. A P value of <0.05 was considered to be statistically significant.

Results

In the first series of experiments, baseline MAP and plasma AVP levels did not differ among the groups (Table 1). Baseline heart rate was also the same among the groups: 382 ± 32 (artificial CSF), 395 ± 37 (ET-1), 389 ± 51 (BQ1223), and 380 ± 52 bpm (ET-1 plus BQ123). Injection of ET-1 into the SFO resulted in a significant increase in heart rate (25 ± 10 bpm), compared with a decrease associated with artificial CSF (–5 ± 9 bpm) ($P < 0.05$). MAP and plasma AVP also increased significantly (Table 1). BQ123 alone did not change MAP or plasma AVP values but inhibited the effects of ET-1. Likewise, BQ123 did not itself alter the heart rate (–2 ± 7 bpm) but prevented the increase induced by ET-1 (–2 ± 3 bpm) ($P < 0.05$ vs. ET-1). The sites of successful injection into the SFO are shown in Figure 1A.

Similarly, baseline parameters in the second series of experiments were similar among the groups (Table 2). Following a 15-min stabilization period after injections into the PVN, heart rate and MAP had returned to values no different from those at baseline (data not shown). Figures 2 and 3 depict the changes in MAP and plasma AVP, respectively, after injection of either artificial CSF or ET-1 into the SFO in rats with bilateral PVN injections of either saline, CNQX, or MK801. PVN injections with saline vehicle did not alter the pressor or AVP response to SFO injection of ET-1. Injection of the PVN with CNQX followed by injection of artificial CSF into the SFO did not result in significant changes in MAP (–5 ± 2 mm Hg), heart rate (–30 ± 13 bpm), or plasma AVP (1.5 ± 1.2 pg/ml), nor did PVN injection with MK801: MAP, 5 ± 5 mm Hg; heart rate, 7 ± 25 bpm; and plasma AVP, –0.8 ± 1.5 pg/ml. Of note, however, the AMPA antagonist CNQX blocked both the pressor response and the increase in plasma AVP in response to ET-1, but the NMDA blocker did not.

Discussion

The major finding of the present study is that AMPA receptors but not NMDA receptors within the magnocellular area of the PVN mediate the pressor and AVP secretory response to ET-1 acting at the SFO. These results are consistent with results of our earlier work showing that bilateral lesions of this region of the PVN prevent the increase in both systemic arterial pressure and plasma AVP in sinoaortic denervated rats when ET-1 is given intra-

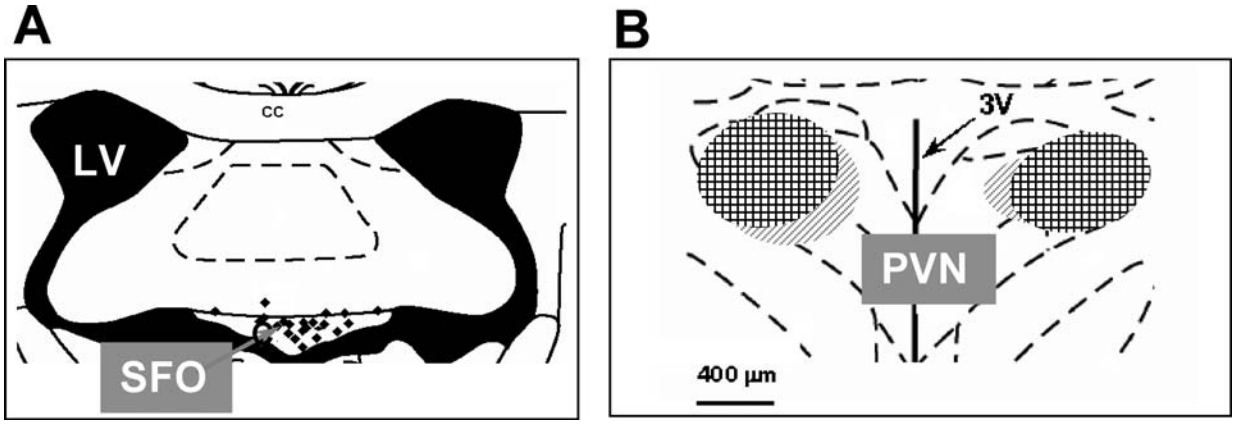

Figure 1. (A) Diagram of a coronal section through the subfornical organ (SFO); ♦ indicates the sites of injection. (B) Diagram of a coronal section through the paraventricular nucleus (PVN). The hatched areas indicate the site of 90% of the injections. Some injections extended medially into the parvicellular region (striped areas). LV, left ventricle; CC, corpus callosum; 3V, third ventricle.

Table 2. Baseline Parameters Before PVN Injection[a]

PVN injection	SFO injection	No. of rats	MAP (mm Hg)	Heart rate (bpm)	AVP (pg/ml)
Vehicle	aCSF	6	119 ± 4	382 ± 13	1.8 ± 0.4
Vehicle	ET-1	8	117 ± 3	419 ± 8	1.7 ± 0.2
CNQX	aCSF	8	120 ± 4	389 ± 18	2.9 ± 1.0
CNQX	ET-1	8	123 ± 3	426 ± 12	1.9 ± 0.4
MK801	aCSF	5	124 ± 4	378 ± 26	2.5 ± 0.4
MK801	ET-1	5	123 ± 4	391 ± 14	1.7 ± 0.5

[a] Values are mean ± SE.

cerebroventricularly (8, 9). The current experiments used direct injections into the SFO, thereby substantially focusing the scope of ET-1 actions and limiting the potential effects of ET-1 at other circumventricular organs that might be involved in AVP release (structures anteroventral to the third ventricle) or sympathetic efferent activity (area postrema). Thus, we can conclude with greater confidence that ET-1 produces these effects by acting at the SFO.

The SFO possesses abundant ET receptors, particularly the ET_A receptor subtype (18). Our findings strongly implicate an ET_A receptor mechanism in both the hemodynamic response and AVP secretion but do not exclude a role for ET_B receptors in this nucleus. The SFO, in turn, sends out two major efferent projections: precommissural bundles to the anteroventral area surrounding the third ventricle, including the median preoptic nucleus, the organum vasculosum of the lamina terminalis, and supraoptic nuclei; and postcommissural fibers, some of which pass through the PVN to terminate in the supraoptic nuclei, and others that terminate in the PVN itself (19, 20). The SFO lies outside the blood brain barrier and is, therefore, capable of being influenced by agents within the systemic circulation. More than half of the SFO neurons antidromically identified as projecting to the PVN exhibit excitatory responses to intravenously administered ET-1. After electrolytic destruction of the SFO, the proportion of phasically firing cells in the PVN that were responsive to systemic ET-1 decreased dramatically (2). Of note, ET-1 injection into the PVN does not change arterial pressure or plasma AVP (32), thus eliminating a direct action of ET-1 on PVN neurons to elicit these responses. Rather, these studies support a model where the PVN acts as a relay for ET-1–stimulated pathways projecting from the SFO to evoke AVP release from the neurohypophysis and sympathetic efferent activity from caudal cardiovascular centers.

Electrical stimulation of PVN neurons elicits an increase in efferent sympathetic activity and a concurrent increase in heart rate and arterial pressure (33, 34). NMDA and non-NMDA receptors are expressed in PVN (23, 34). Importantly, glutamate injections into the PVN activate the sympathetic nervous system (14). Glutamatergic signaling is one of the major excitatory inputs to the somata and dendrites of magnocellular neurons within the PVN (24, 29). Electrophysiological data support a role for both NMDA and non-NMDA receptors in the activation of vasopressinergic neurons (22, 27, 28), and both NMDA and non-NMDA receptors have been shown to modulate AVP

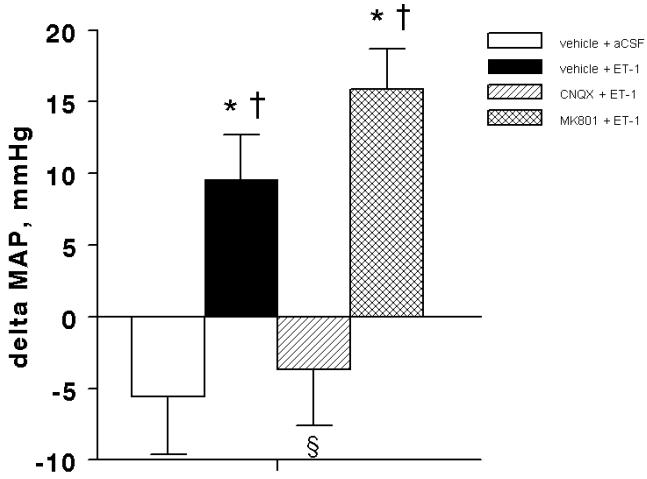

Figure 2. Change mean arterial pressure (MAP) after microinjection of vehicle (saline), CQNX, or MK801 into bilateral PVN followed 10 mins later with either artificial CSF (aCSF) or ET-1 microinjected into SFO. Values are mean ± SE ($n = 6$, 8, 11, and 8). $*P < 0.02$ vs. baseline (not shown); $\dagger P < 0.05$ vs. vehicle + aCSF; and $\S P < 0.05$ vs. vehicle + ET-1.

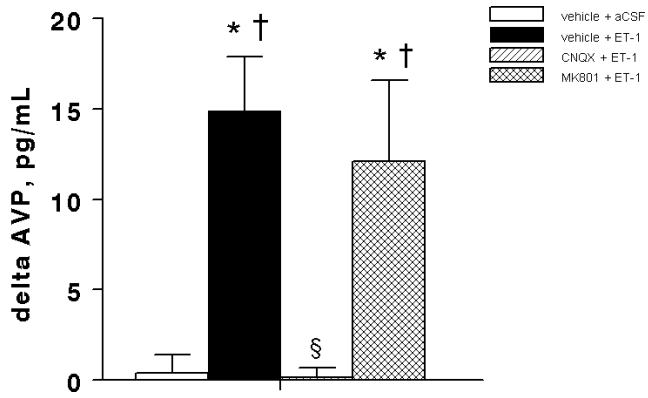

Figure 3. Change in plasma AVP levels after microinjection of either vehicle (saline), CQNX, or MK801 into bilateral PVN followed 10 mins later with either artificial CSF (aCSF) or ET-1 microinjected into SFO. Values are mean ± SE ($n = 6$, 8, 11, and 8). $*P < 0.02$ vs. baseline (not shown); $\dagger P < 0.05$ vs. vehicle + aCSF; and $\S P < 0.001$ vs. vehicle + ET-1.

release to osmotic stimuli (25, 26). In particular, AMPA receptor antagonism prevented the antidiuretic response to intracerebroventricular administration of NMDA (29). Recently, we demonstrated that an NMDA receptor, which was inhibited by MK801, mediated ET_B receptor–induced AVP release by hypothalamoneurohypophysial explants (16). However, that study could not address whether an ionotropic glutamatergic receptor mediates the effect of ET-1 on SFO, because neither the SFO nor the PVN is contained within the explant tissue.

Our results showing that both the pressor and AVP secretory responses were suppressed by an inhibitor of AMPA receptors, but not NMDA receptors, were somewhat surprising but not unexpected. NMDA receptors are both ligand gated and voltage sensitive. Magnesium ion blocks the ion channel. When glutamate acts on the non-NMDA receptors to depolarize the membrane, magnesium exits the channel and permits activation of NMDA receptors. This voltage sensitivity of the NMDA receptors contributes to the bursting activity of vasopressinergic neurons and facilitates AVP release (25, 27, 28, 35). Once released into the PVN from axonal projections emerging from SFO, glutamate may act on NMDA or non-NMDA receptors. Our data suggest that activation of non-NMDA, CNQX-sensitive AMPA receptors is required for optimal signaling from PVN neurons projecting to the caudal medulla that regulates systemic hemodynamics, as well as to anterior hypothalamic sites that secrete AVP. The observation that MK801 did not inhibit the pressor or AVP responses supports the concept that AMPA-mediated effects on AVP and blood pressure are independent of NMDA receptor activation in the PVN. This interaction of NMDA and AMPA receptors has also been observed with osmotic stimulation of AVP *in vitro* (25).

In summary, these findings support the conclusion that both the pressor response and AVP secretion in response to ET-1 acting at the SFO are mediated by a non-NMDA, most likely an AMPA glutamatergic receptor, within the PVN.

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