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HYPOTENSION TO CENTRAL ENDOTHELIN-1: EFFECT OF GLUTAMATE RECEPTOR BLOCKADE

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Summary: Endothelin-1 (ET-1) produces hypotension via an action at glutamate-sensitive medullary cardiovascular sites. Here, we used excitatory amino acid (EAA) receptor antagonists to examine the possible role of an endogenous EAA in this neural action of central ET-1. ET-1 (3 pmol) applied to the IV ventricle of anesthetized, artificially ventilated rats elicited a sustained decrease in blood pressure (27 ± 6%). Pretreatment with two EAA receptor antagonists, APV and CNQX (or MK-801 and CNQX), significantly attenuated the hypotension to central ET-1 (11 ± 4%). Since these antagonists do not interact with endothelin receptors, we conclude that release of an endogenous EAA may contribute to the hypotensive action of central ET-1.

Endothelins are potent vasoconstrictor peptides (30) and are widely distributed in mammalian tissues including the central nervous system (3, 10). In the anesthetized, ventilated cat, rabbit and rat, central ET-1 elicits a prominent, sustained depressor response, sometimes accompanied by a transient pressor component (8, 9, 12, 13, 17, 24, 26). This hypotensive effect of central ET-1 (1-10 pmol) is neuronally mediated (12, 13, 24, 26).

Various brainstem cardiovascular nuclei have high densities of binding sites for both ET-1 (3) and EAAs (6). Several medullary cardiovascular sites (e.g., nucleus tractus solitarii and rostroventrolateral medulla) are sensitive to both ET-1 and L-glutamate (13, 17, 24). Like ET-1 (13, 24), the EAA agonists glutamate, NMDA and AMPA produce depressor responses (13, 17, 18) while kainic acid elicits both pressor and depressor responses from these sites (19, 27, 32). The foregoing raised the possibility that an EAA may be involved in the central ET-1-induced responses. To test this hypothesis, we examined the effect of EAA receptor blockade on central ET-1-induced hypotension. In order to block multiple EAA receptors that mediate cardiovascular responses from the brainstem (19, 27), we used combinations of EAA receptor antagonists.

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Abbreviations: **4CV** IV cerebral ventricle; **AMPA** (R,S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; **APV** D,L-2-amino-5-phosphonovaleric acid; **CNQX** 6-cyano-7-nitroquinoxaline-2,3-dione; **EAA** excitatory amino acid; **ET-1** Endothelin-1; **MAP** mean arterial blood pressure; **MK-801** (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine hydrogen maleate; **NMDA** N-methyl-D-aspartate.

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Materials and Methods

Male Sprague-Dawley rats (275-450 g; Taconic Farms, New York) were anesthetized with thiobutabarbital sodium (Inactin) 100 mg/kg, i.p., tracheostomized, and artificially respired using a rodent ventilator (Ugo Basile, Italy). The left femoral vein and artery were cannulated. Arterial blood pressure was recorded via a Gould P23-ID pressure transducer on a Grass (P7) or a Gould (series 3800) chart recorder. Heart rate (HR) was monitored by means of a tachograph triggered by the arterial pressure pulse. Rectal temperature of the animal was maintained between 36.5° and 37.5° C. by a thermistor-controlled infrared heat lamp and a heat pad. The animal was fixed in a stereotaxic frame (David Kopf) and, as described previously (13), the 4CV was exposed for direct drug application to its caudal surface. All data (means ± S.E) are presented as percent of, or percent change from, the baseline value. Analysis of variance and Newman-Keuls tests were used to examine significant differences within and between treatment groups. Statistical significance was indicated by a P value of 0.05 or less.

Drugs used and their sources were as follows: Inactin (Byk Gulden Constanz); ET-1 (Peptides Institute, Inc.); kainic acid, NMDA (Sigma); APV, AMPA, CNQX, MK-801 (RBI). CNQX (10 mM) was dissolved in 0.1N sodium hydroxide and the pH adjusted to 8. Solutions of APV (100 mM) and MK-801 (10 mM) were made in distilled water. Solutions of ET-1 (1 μM), NMDA (1 mM) and AMPA (1 mM) were made in saline.

Experimental Protocol

A 30-min stabilization period followed completion of surgery. Five series of experiments were conducted as follows: (i) Doses of EAA antagonists required to block central EAA receptors were determined; (ii) blood pressure responses to central ET-1 (3 pmol; 3 μl) were obtained; (iii) effects of central ET-1 were tested after treatment with a single EAA antagonist (either APV, CNQX or MK-801); iv) effects of central ET-1 were tested after treatment with a combination of APV and CNQX (or MK-801 and CNQX); the antagonists were given 15 min apart; and (v) intravenous ET-1-induced responses were examined following i.v. administration of high doses of MK-801 and CNQX. In all cases, ET-1 was applied to 4CV 15 min after administration of the EAA receptor antagonists. Control experiments were also done with equal volumes of vehicle applied to the 4CV which did not produce any significant effects.

Results

Determination of EAA receptor antagonist doses

Blockade of central EAA agonist-induced depressor responses was used as a guide to determine effective doses of CNQX, APV and MK-801. Since kainic acid did not consistently evoke a depressor response, only NMDA and AMPA (3 nmol each) were used as agonists. In different experiments, responses to these agonists were tested before and after administration of a particular EAA receptor antagonist. Consistent with other reports (11, 15), short-lasting increases in blood pressure were elicited by each antagonist. Therefore, blood pressure was allowed to return to baseline (15 min) before responses to NMDA or AMPA were tested. Since desensitization can occur with frequent agonist applications, sufficient time (at least 30 min) was allowed between test responses.

Figure 1 depicts the effects of APV and CNQX on agonist-induced depressor responses. APV at a dose of 5 μmol (N=4) abolished the depressor response evoked by NMDA without affecting that elicited by AMPA (N=4). On the other hand, 100 nmol of CNQX (N=4) significantly attenuated the depressor response to AMPA without affecting the response to

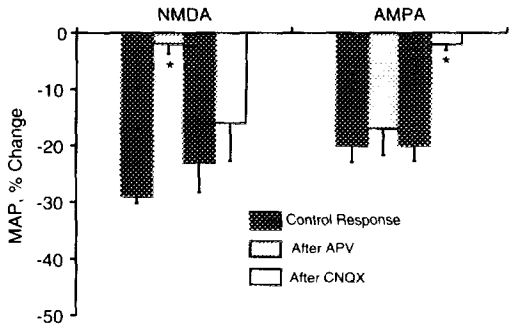

Figure 1. Depressor responses to NMDA (3 nmol) and AMPA (3 nmol) before and after treatment with various EAA receptor antagonists. All agents were applied to the surface of the IV cerebral ventricle. Responses to NMDA or AMPA were obtained 30 min before and 30 min following APV (5 μmol) or CNQX (100 nmol). For each set, control (untreated) response is shown to the left of the test (treated) group. All data (means ± S.E.) are expressed as percent change from preinjection (baseline) values. Baseline values in all cases ranged between 108 and 120 mm Hg and were not significantly different from one another. Asterisk (*) denotes significant difference from control response.

NMDA (N=3). As with APV, depressor responses to NMDA were also selectively blocked by MK-801 at a dose of 1 μmol. At these doses, each antagonist maintained the relatively selective EAA receptor blockade for up to 60 min. In other experiments, we found that treatment with a combination of CNQX and APV (N=3; applied to the 4CV at 15 min intervals in random order) abolished the depressor responses to both AMPA and NMDA. Similarly, treatment with a combination of CNQX and MK-801 (N=3) blocked the depressor responses to both EAA agonists. Based on these results, doses of antagonists used in subsequent experiments with ET-1 were: APV=5 μmol; CNQX=100 nmol; MK-801=1 μmol.

Cardiovascular responses to central ET-1

A 3 pmol dose of ET-1 was used in all experiments since this dose consistently produces sustained hypotension (13). Time course of MAP, expressed as percent change from baseline, is depicted in figure 2. ET-1 produced a gradual decrease in MAP which peaked (27 ± 6%) at 15 min and was significant from 5 to 30 min (Fig. 2). MAP returned to baseline levels by 60 min. Changes in heart rate were small (±10%) and not significant (baseline=405 ± 11 beats/min). Application of saline to the 4CV did not produce any significant changes in MAP. Baseline values for MAP in various groups and number of experiments are given in table 1.

Central ET-1-induced hypotension: effect of treatment with a single EAA antagonist

The hypotensive response to central ET-1 was examined in different groups of rats pretreated with either APV, CNQX or MK-801. In each case, ET-1 was applied to the 4CV 15 min after administration of antagonist. Results are depicted in figure 2A. In rats treated with APV, the ET-1-induced decrease in MAP was delayed: although no decrease in MAP was seen for up to 5 min, hypotension occurred subsequently whose magnitude was similar to that of the untreated group (Fig. 2A). Treatment with CNQX or MK-801 also produced results similar to APV: delayed but significant hypotension occurred after central application of ET-1 (Fig. 2A).

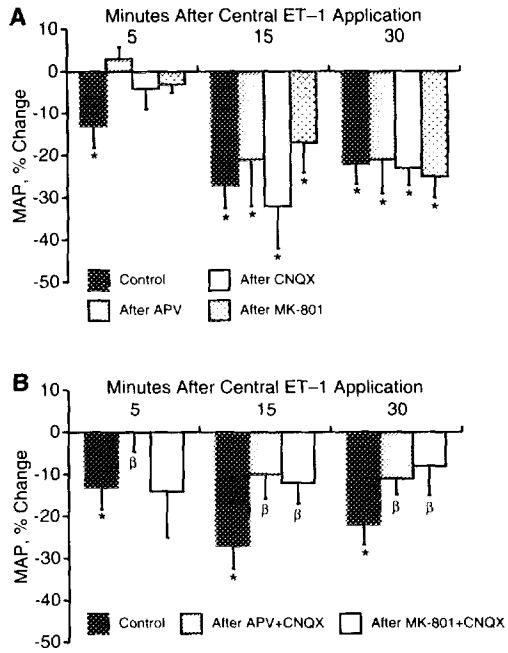

Figure 2. Central ET-1-induced hypotension: Effect of EAA receptor antagonist treatment. **A**: Treatment with a single antagonist -- APV, CNQX or MK-801. **B**: Treatment with combination of two antagonists: APV + CNQX or MK-801 + CNQX. Decreases in mean blood pressure at various time points are shown following application of ET-1 to the IV ventricle in untreated (control) and EAA receptor antagonist treated groups. Data (means ± S.E.) are expressed as percent change from preinjection (baseline) values. Baseline values are presented in table 1. Asterisk (*) denotes significant difference from baseline value; β, significant difference from corresponding time-point in untreated group.

Table 1

Baseline blood pressure in various groups

Test Group	Treatment	MAP (mm Hg) Before Treatment	MAP (mm Hg) After Treatment
ET-1	None	110 ± 4 (13)	--
ET-1	APV	110 ± 4 (5)	119 ± 3 (5)
ET-1	CNQX	107 ± 2 (3)	115 ± 0 (3)
ET-1	MK-801	115 ± 9 (5)	113 ± 9 (5)
ET-1	APV + CNQX	105 ± 11 (8)	103 ± 6 (8)
ET-1	MK-801 + CNQX	95 ± 4 (5)	94 ± 5 (5)
Saline	None	95 ± 5 (5)	--

Post-treatment values for MAP are those seen 15 min after each treatment. All agents were applied to the 4CV. Number of experiments is given in parentheses.

Central ET-1-induced hypotension: effect of treatment with two EAA antagonists

The effects of ET-1 were examined following treatment with a combination of APV and CNQX. The two antagonists were applied at 15 min intervals followed 15 min later by ET-1. As shown in figure 2B, in APV and CNQX treated rats, ET-1 did not produce a significant decrease in MAP; only small, non-significant decreases in MAP (peak decrease=11 ± 4%) were observed at 15-30 min whose magnitude was significantly less than that of untreated rats. In another set of rats, effects of ET-1 were examined after pretreatment with MK-801 and CNQX. Following this treatment, central ET-1 again did not produce a significant decrease in MAP (Fig. 2B). Also, the magnitude of the decrease in MAP was significantly less than that in untreated rats.

Cardiovascular responses to intravenous ET-1: effect of treatment with two EAA antagonists

In order to determine if the EAA receptor antagonists were interacting with endothelin receptors, we examined the effects of high doses of MK-801 and CNQX on blood pressure responses to intravenously administered ET-1. At a dose of 300 pmol/kg, i.v., ET-1 produced the characteristic (30) sharp fall in MAP (baseline MAP=107 ± 6; peak fall=38 ± 6%; N=3) followed by a gradual, sustained rise (peak increase=20 ± 1%). This biphasic response to ET-1 remained unaffected following i.v.administration of MK-801 (75 μmol/kg) and CNQX (15 μmol/kg). Thus, ET-1 (300 pmol/kg, i.v.; N=3), administered 15 min after the above treatment, induced a fall in MAP of 40 ± 3% (baseline MAP=106 ± 6) followed by an increase of 17 ± 3%. Brief, inconsistent changes in MAP were noted with MK-801 and CNQX.

Discussion

Previous work showed that centrally administered ET-1 evokes hypotension via a neuronally mediated action (8, 12, 13, 24, 26). The present study indicates that this hypotension may be due, in part, to the release of an EAA by central ET-1. This conclusion is based on the observation that blockade of EAA receptors attenuated central ET-1-induced hypotension.

We found that blockade of a single class of EAA receptors delayed but did not prevent central ET-1-induced hypotension. The selectivity of the antagonists was demonstrated by our results with EAA agonists: CNQX blocked the depressor response to AMPA but not that to NMDA; similarly, APV (or MK-801) selectively blocked the depressor response evoked by NMDA. Within the brainstem, multiple EAA receptors mediate the depressor responses to EAAs (11, 18, 19, 27). Therefore, we used combinations of antagonists since broad-spectrum EAA receptor antagonists (e.g., kyneuranic acid) are relatively weak (29). Following blockade of multiple classes of brainstem EAA receptors (with APV + CNQX or MK-801 + CNQX) central ET-1 did not produce significant hypotension. These data suggest that release of an EAA may contribute to the central ET-1-induced hypotension since EAA receptor antagonists do not block endothelin receptors (see below). This conclusion is consistent with the finding that ET-1 releases an EAA from cerebellar granule cells (20).

Available evidence indicates that EAA receptor antagonists used in this study do not block endothelin receptors. Radioligand binding assays demonstrate that MK-801 does not interact with endothelin binding sites in a variety of tissues (4). Further, studies in our laboratories

indicate that neither CNQX nor APV affects the binding of [^{125}I]ET-1 to cerebral or cardiac membranes (unpublished observations). Also, in the present study, peripheral cardiovascular actions of ET-1 were unaffected by high doses of MK-801 and CNQX. These data, together with the fact that central and peripheral endothelin receptors are homologous (1, 25), indicate that attenuation of ET-1-induced hypotension is not due to blockade of endothelin receptors.

There is evidence to suggest that EAAs play a role in central cardiovascular regulation (11, 15) and that EAA receptor antagonists inhibit the baroreceptor reflex (16). In contrast, ET-1 is reported to sensitize the baroreceptor reflex (14). Therefore, it can be argued that a physiologic antagonism may counteract the ET-1-induced hypotension. However, in our study, EAA antagonist-induced increases in blood pressure were brief and ET-1 was always administered only after the blood pressure had returned to baseline levels. Thus, a physiologic antagonism is unlikely, but cannot be excluded.

Our recent studies indicate that central neuronal ET_A receptors mediate the hypotension (26). The foregoing, together with the *in vitro* demonstration of EAA release by ET-1 from neurons (20), suggests a direct neuronal action of ET-1 to release EAAs. On the other hand, ET-1 at higher doses (>30 pmol) can cause cerebral ischemia (12, 21) which can lead to the release of EAAs (23); this possibility cannot be ruled out. In this study, EAA antagonists attenuated, but did not abolish, the central ET-1-induced hypotension. Whether excessive release of an EAA overcomes the antagonism or additional mechanisms are involved in this paradigm is unclear. A recent report indicates that activation of a fourth class of EAA receptors, the metabotropic glutamate receptor, in the brainstem also evokes hypotension (29). Selective and potent antagonists for this receptor are currently unavailable (29).

In conclusion, our study provides indirect evidence to suggest that central ET-1-induced hypotension is due, in part, to the release of an endogenous EAA. Both endothelins and EAAs have been implicated in neurodegenerative processes. ET-1 produces cerebral ischemia (21) and is implicated in the production of cerebral vasospasm associated with subarachnoid hemorrhage (2, 5, 7, 22). Excessive EAA release occurs during cerebral ischemia (23). Whether or not a direct interaction between ET-1 and EAAs contributes to the pathogenesis of cerebral vasospastic disorders remains for future investigations.

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