Excitatory Gastric Motor and Cardiovascular Effects of Endothelins in the Dorsal Vagal Complex are Mediated Through ET_A Receptors

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Accepted for publication April 24, 1997

ABSTRACT

We have shown recently that intracisternal administration of endothelin-(ET)-1 and ET-3 evokes increases in gastric motor function and arterial blood pressure. The aim of our study was to investigate whether the dorsal vagal complex (DVC) is a medullary site of action for the gastric motor and cardiovascular effects of ET-1 and to identify the ET receptor subtype through which these effects are mediated. ET-1 (0.1–40 pmol/site) and ET-3 (1 and 100 pmol/site) were microinjected into the DVC of α-chloralose anesthetized rats, while monitoring intragastric pressure, contractile activity of greater curvature longitudinal and pyloric circular smooth muscle, arterial blood pressure and heart rate. ET-1, at doses of 0.1 to 40 pmol, increased intragastric pressure and, at doses of 10 and 40 pmol, increased pyloric contractile activity and arterial blood pressure. The increases in gastric motor function, but not the hypotension, induced by ET-1 (10 pmol) in the DVC were completely abolished by bilateral vagotomy. Spinal cord transection prevented increases in arterial blood pressure evoked by ET-1 (40 pmol). Because only the highest dose of ET-3 (100 pmol), microinjected into the DVC, increased intragastric pressure and pyloric contractile activity and no consistent changes in cardiovascular functions were noted, we hypothesized that the gastric motor and hypertensive responses to endothelins in the DVC are mediated via ET_A receptors. This was supported by the observation that a selective ET_A receptor antagonist, cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu) (BQ-123; 400 pmol), microinjected into the DVC 15 min before ET-1 (10 pmol) or ET-3 (100 pmol), completely blocked the gastric motor and cardiovascular responses to endothelins. We conclude that endothelins act in the brainstem at the level of the DVC to increase intragastric pressure and gastric contractile activity via a vagally mediated pathway and that both the gastric motor and hypertensive effects of endothelins in the DVC are mediated through ET_A receptors.

ET, produced by the endothelium, brain and gastrointestinal tract, has a wide spectrum of biological actions in a variety of tissues (Masaki et al., 1992) and includes three different isoforms, namely ET-1, ET-2 and ET-3 (Inoue et al., 1989). Thus far, only two cDNA clones encoding ET receptors have been identified in mammalian tissues; the ET_A receptor that exhibits a higher affinity for ET-1 and ET-2 than for ET-3 and the ET_B receptor that is equally sensitive for all ET isoforms (Masaki et al., 1994). Endothelin isoforms have been implicated in the modulation of both vascular and nonvascular (e.g., intestinal) smooth muscle contractile activity (Masaki et al., 1992; Rae et al., 1995). However, there is also growing evidence that ET has neuromodulatory activities. Autoradiographic studies have demonstrated high-affinity binding sites for ET in the brain (Jones et al., 1989; Koseki et al., 1989) and immunohistochemical, Northern blot and in situ hybridization techniques have identified the presence of ET and its mRNA in neurons (Lee et al., 1990; MacCumber et al., 1989). Endothelin isoforms are involved in the central control of autonomic function (Gulati et al., 1992; Hashim and Tadepalli, 1992; Mosqueda-Garcia et al., 1992, 1995a) and we have recently reported that both ET-1 and ET-3 increase gastric motor and cardiovascular function when applied to the dorsal surface of the medulla oblongata (Krowicki and Hornby, 1996).

The vagus nerve provides parasympathetic control of the gastrointestinal system and the heart. Gastrointestinal (Ka- lia and Mesulam, 1980; Krowicki and Hornby, 1995a) and some cardiac (Izzo et al., 1993; Kalia and Mesulam, 1980)
parasympathetic preganglionic neurons originate in the dor-
sal motor nucleus of the vagus, whereas baroreceptor and
gastrointestinal afferent input terminates in the nTS (Agar-
wal and Calaresu, 1992; Miselis et al., 1991). The dorsal
motor nucleus of the vagus and nTS are often considered as
a DVC, which is a target for hypothalamic and hindbrain
efferents containing both “classical” neurotransmitters and
neuropeptides. The DVC is also a potential site of action for
blood-borne substances. This is because the caudal nTS has
fenestrated capillaries and enlarged perivascular spaces
(Gross et al., 1980) that permit entry of large serum proteins
(Broadwell and Sofroniew, 1993). In addition, there are neu-nonal connections between the DVC and the area postrema, a
circumventricular organ on top of the fourth ventricle (Sha-
piero and Miselis, 1985).

Because autoradiographic studies have documented the
presence of ET-1 binding sites in the DVC (Kohzuki et al.,
1991; Koseki et al., 1989; van den Buuse and Itoh, 1993), we
investigated whether the DVC is a medullary site for gastric
motor and cardiovascular effects of ET and tried to identify
the ET receptor subtype through which these effects are
mediated.

Materials and Methods

Animals. Male Sprague-Dawley rats (215–410 g; Charles River
Laboratories, Wilmington, MA) were used in this study, which was
approved by the LSUMC Institutional Animal Care and Use Com-
mitee. Food was withheld 12 hr before experiments but there was
free access to drinking water.

Surgery. The animals were initially anesthetized with ketamine
and xylazine mixture (i.m., 50 and 5 mg/kg, respectively) and separ-
ate cannulae were placed in left femoral artery (for blood pressure
recording) and vein, then α-chloralose (i.v., 80 mg/kg) was adminis-
tered 25 min later. If needed, urethane (i.v., 600 mg/kg) or xylazine
(i.v., 2.5 mg/kg) were used to maintain full surgical anesthesia in the
presence of α-chloralose. After a tracheotomy, the rats were artifi-
cially ventilated (tidal volume 1 ml/100 g; rate 60/min) using a small
animal respirator (Kent Scientific Corp., Litchfield, CT), because centrally administered ET-1 produces apnea in α-chloralose-anes-
ethetized rats (Fuxe et al., 1989a). Intragastric pressure was continu-
ously recorded using a latex balloon placed in the stomach. Addi-
tionally, two small strain gauges were mounted on the surface of the
stomach for monitoring of circular smooth muscle contractile activity
of the pyloric region and longitudinal smooth muscle of the greater
curvature of the stomach (Krowicki and Hornby, 1993). A separate
catheter was placed in the left femoral artery and connected to a
pressure transducer (Viggo-Spectramed, model P23XL, Oxnard, CA)
and polygraph (model 7E, Grass Instrument Co., Quincy, MA) for
direct measurement of blood pressure. Heart rate was monitored by
a tachograph triggered by the arterial pressure pulse (model 7P4H,
Grass Instrument Co., Quincy, MA). On some animals, additional
surgical procedures were performed. Bilateral vagotomy was per-
formed in five animals at the midcervical level. Briefly, the vagi were
carefully separated from the left and right common carotid arteries
and silk snare were loosely placed around them, then vagotomy was
achieved by avulsion. Transection of the cervical spinal cord was
performed in four animals at the level of the medullospinal transi-
tion region. To prevent activation of nociceptive reflexes, 0.5 ml of
2% lidocaine HCl (Butler, Columbus, OH) was injected with a 25-gauge
needle directly into the exposed spinal cord in several locations, then
0.5 cm of the cord was excised to ensure complete interruption of
spinal efferents. These acute, terminal procedures were performed in
the presence of full surgical anesthesia. Rectal temperature was
maintained between 37.0 and 37.5°C.

Microinjections. Animals were then placed prone in a stereo-
taxic frame with the tooth bar set at −6.5 mm. The caudal floor of the
fourth cerebral ventricle and the surrounding structures of the dor-
sal medulla oblongata were exposed through an incision of the dura
mater and the arachnoid membrane. Under visual control through a
stereoscopic eyeglass magnifier (Stereomax A. Siebert, Wetzler, Ger-
many), five-barreled glass micropipette tips (FHIC, Brunswick, ME;
20 µm total external tip diameter) were stereotaxically placed in the
right DVC (coordinates: 0.5 mm rostral to the obex, 0.5 mm lateral
from the midline and 0.45–0.5 mm down from the surface). Minor
adjustments to these coordinates for the final injections within the
DVC were made by identifying the most sensitive location for gastric
excitatory effects of L-GLU (7.5 nmol). In our experience, L-GLU
increases intragastric pressure and gastric smooth muscle contrac-
tility when the micropipette tip is placed in the DVC, in the region of
the dorsal motor nucleus of the vagus (Krowicki et al., 1997). All
microinjections were delivered in a volume of 30 nl (30 psi) using a
pneumatic pico-pump model PV 830 (World Precision Instruments,
New Haven, CT). At the end of each experiment, 1 to 2% pontamine
sky blue was microinjected into the DVC in a volume of 30 nl. The
injection sites were confirmed microscopically in the fixed counter-
stained sections, as described in detail elsewhere (Krowicki and
Hornby, 1993).

Drugs. Endothelin-1, ET-3 and a selective ET₄ receptor antago-
nist BQ-123 (Hashim and Tadepalli, 1992), purchased from the
American Peptide Co. (Sunnyvale, CA) or Peninsula Laboratories,
Inc. (Belmont, CA), were dissolved in 0.9% NaCl with 0.1% bovine
serum albumin radioimmunoassay grade.

Data analysis. The area of the response in intragastric pressure
for each treatment was calculated using a microcomputer-based
imaging system (Imaging Research, Ontario, Canada). For this pur-
pose, the baseline was extended across the period of the response to
the point at which intragastric pressure had returned to baseline,
and the area of the response was calculated as the area enclosed
between the baseline and the curve of the response (Krowicki and
Hornby, 1995b). For peak response in intragastric pressure (maxi-
mum difference from baseline), pyloric and greater curvature minute
motility indexes, heart rate and blood pressure, the changes from
baseline were calculated. Minute motility index was calculated for 2
min before and after microinjection according to Ormsbee and Bass
(1976), as reported previously (Krowicki and Hornby, 1993). Blood
pressure is expressed as MAP and was calculated by adding one-
third of the pulse pressure to the diastolic pressure.

The differences between groups were assessed by paired t test or
by one-way analysis of variance followed by Student-Newman-Keuls
test. Because heart rate and MAP data were not normally distrib-
uted, Kruskall-Wallis one-way analysis of variance with subsequent
Dunn’s test were applied. Values of P < .05 were considered to be
statistically significant.

Results

Gastric motor and cardiovascular effects of ET-1 in the
DVC. The effects of vehicle and ET-1, microinjected into the
DVC at doses of 0.1, 1, 10 and 40 pmol, on gastric motor
function are shown in figure 1. Endothelin-1 evoked in-
creases in peak intragastric pressure at all doses. However,
changes in the total area of the response achieved statistical
significance only at doses of 10 and 40 pmol. Similarly, pylor-
ic contractile activity increased in response to ET-1 at doses
of 10 and 40 pmol. The changes in greater curvature contrac-
tile activity attained statistical significance only at the high-
est dose of ET-1 used in the study (40 pmol). Table 1 shows
the effect of ET-1 (0.1–40 pmol) in the DVC on heart rate and
MAP. Significant increases in blood pressure resulted after
ET-1 in the DVC at doses of 10 and 40 pmol; however, the
Effects of vehicle (N = 17) or ET-1 at a dose of 0.1 pmol (N = 7), 1 pmol (N = 6), 10 pmol (N = 10) and 40 pmol (N = 7), microinjected into the DVC, on intragastric pressure (PRIGP, peak response; ARIGP, area of the response), pyloric circular muscle (PCA) and greater curvature longitudinal muscle (GCCA) contractile activity. Data are mean (bar = S.E.) changes from baseline for number of animals indicated under the x axis. * Statistically significant when compared with corresponding vehicle using Kruskal-Wallis one-way analysis of variance with subsequent Dunn’s test.

### TABLE 1

Peak changes in heart rate and MAP induced by microinjection of vehicle, ET-1 or ET-3 into the DVC; the number of rats in each group is given in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Median</th>
<th>25%</th>
<th>75%</th>
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<tr>
<td>Heart rate</td>
<td>Vehicle (15)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-1 0.1 pmol (7)</td>
<td>-10</td>
<td>-10</td>
<td>-13</td>
</tr>
<tr>
<td></td>
<td>ET-1 1 pmol (6)</td>
<td>-8</td>
<td>-20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-1 10 pmol (9)</td>
<td>-10</td>
<td>-13</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>ET-1 40 pmol (7)</td>
<td>40</td>
<td>28</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Vehicle (6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-3 1 pmol (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-3 10 pmol (6)</td>
<td>-3</td>
<td>-20</td>
<td>10</td>
</tr>
<tr>
<td>MAP</td>
<td>Vehicle (16)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-1 0.1 pmol (7)</td>
<td>5</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>ET-1 1 pmol (6)</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>ET-1 10 pmol (9)</td>
<td>10*</td>
<td>8</td>
<td>16</td>
</tr>
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<td></td>
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<td>64</td>
</tr>
<tr>
<td></td>
<td>Vehicle (8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-3 1 pmol (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ET-3 10 pmol (8)</td>
<td>0</td>
<td>-5</td>
<td>5</td>
</tr>
</tbody>
</table>

* Statistically significant when compared with corresponding vehicle using Kruskal-Wallis one-way analysis of variance with subsequent Dunn’s test.

The heart rate response was very variable and did not attain statistical significance. In two of nine animals microinjected into the DVC with ET-1 at a dose of 40 pmol, tremendous fluctuations in heart rate and MAP occurred after microinjection. Therefore, the data from these animals were not included in calculations. The locations of the tip of the micropipette in the animals that received ET-1 at a dose of 10 pmol into the DVC are shown in figure 2.

Tracing recordings from a representative experiment in which ET-1, at doses of 0.1 and 10 pmol, was microinjected into the DVC are shown in figure 3. After microinjection of ET-1 into the DVC at a dose of 0.1 pmol, an increase in intragastric pressure (peak: +3.5 cm H2O; area of the response: 0.32 cm2) occurred immediately after injection and returned to baseline within 40 sec (fig. 3A). Little discernable changes in pyloric MMI (−2.0) and heart rate (−10 bpm) as well as an increase in MAP (+5 mmHg) were observed. After ET-1 at a dose of 10 pmol (fig. 3B), an increase in intragastric pressure (peak: +5.0 cm H2O; area of the response: 1.38 cm2) occurred immediately after injection and returned to baseline 2 min later. Increases in MMI of the pyloric (+10) and greater curvature smooth muscle MMI (+7.5), heart rate (+10 bpm) and MAP (+10 mmHg) were noted at this dose in the same animal (fig. 3B).

To assure the lack of neurotoxic effect of ET-1 at a dose of 40 pmol, L-GLU (7.5 nmol) was microinjected into the DVC 30–60 min after ET-1 in two rats. Excitatory gastric motor responses similar to the response to L-GLU prior to ET-1 were observed in these animals (data not shown).

**Fig. 2.** The location of the micropipette tips (triangles) after microinjection of ET-1 (10 pmol) are transposed onto the right DVC, although nuclei are labeled on the left DVC. Sections are drawn by using a drawing tube attachment to a Nikon Labophot microscope and are arranged from most caudal at the top (0.7 mm rostral to the obex) to most rostral at the bottom (1.0 mm rostral to the obex). Abbreviations: AP, area postrema; cc, central canal; DMV, dorsal motor nucleus of the vagus; mlf, medial longitudinals fasciculus; NTS, nucleus of the solitary tract; TS, solitary tract; XII, hypoglossal nucleus. Bar = 500 μ, original magnification × 2.

**Fig. 1.** Effects of vehicle (N = 17) or ET-1 at doses of 0.1 pmol (N = 7), 1 pmol (N = 6), 10 pmol (N = 10) and 40 pmol (N = 7), microinjected into the DVC, on intragastric pressure (PRIGP, peak response; ARIGP, area of the response), pyloric circular muscle (PCA) and greater curvature longitudinal muscle (GCCA) contractile activity. Data are mean (bar = S.E.) changes from baseline for number of animals indicated under the x axis. * Statistically significant when compared with corresponding vehicle using Kruskal-Wallis one-way analysis of variance with subsequent Dunn’s test.
Control microinjections. To ascertain the anatomic specificity of the gastric and cardiovascular responses to microinjections of ET-1 into the DVC, ET-1 was microinjected outside the DVC at a dose of 10 pmol in four animals. The tip of the micropipette was placed lateral to the DVC in the medullary tissue using the following coordinates: 0.5 mm rostral to the obex, 1.6 mm lateral from the midline and 0.5 mm down from the surface. No significant changes in gastric motor or cardiovascular function were observed. However, in all these animals, excitatory gastric and cardiovascular responses to ET-1 were noted in response to microinjection into the DVC at the same dose and volume (table 2).

**BQ-123 and ET-evoked gastric motor and cardiovascular excitation in the DVC.** To determine the ET receptor subtype that mediates the gastric motor and cardiovascular responses to ET-1 in the DVC, an ETA receptor antagonist, BQ-123, was microinjected into the DVC at a dose of 400 pmol 15 min before subsequent microinjection of ET-1 (10 pmol), into the same site in five animals. Microinjection of BQ-123 did not alter baseline gastric motor and cardiovascular function 15 min after injection (table 3). However, BQ-123 completely blocked the gastric motor and cardiovascular responses to ET-1 and ET-3 (table 4). In these animals the effect of microinjection of L-GLU into the site before and after BQ-123 microinjection was also determined. A significant increase in intragastric pressure was observed in response to L-GLU (7.5 nmol) before (peak response: 6.1 ± 1.0 cm H₂O) and after BQ-123 administration (peak response: 4.5 ± 1.7 cm H₂O).

**Vagotomy and spinal cord transection.** The effects of vagotomy and spinal cord transection on the autonomic responses to ET-1 microinjections into the DVC were investigated in five and four animals, respectively (table 5). Before vagotomy, ET-1 (10 pmol) increased intragastric pressure (peak and total area of the response) and MAP. The increases in intragastric pressure were abolished by bilateral vagotomy. Before spinal cord transection, ET-1 (40 pmol) evoked increases in peak intragastric pressure, pyloric contractile activity and MAP. Spinal cord transection completely blocked increases in MAP and slightly attenuated the increase in peak intragastric pressure in response to the peptide. Baseline gastric motor and cardiovascular functions before and
Discussion

The major finding of our study is that the DVC is a site of action for the excitatory gastric motor and cardiovascular responses to endothelins. The gastric motor responses to ET-1 in the DVC are mediated primarily through vagal pathways although the changes in MAP are mediated through sympathetic pathways.

The gastric motor and cardiovascular effects of ET-1 and ET-3 in the DVC were completely blocked by an ETA receptor antagonist, BQ-123. Interestingly, microinjection of BQ-123 alone into the DVC did not alter gastric motor or cardiovascular function in our experiments, although blockade of ET₂ receptors in the nTS with 5-fold higher dose of BQ-123 was reported to decrease blood pressure or, after recovery of the initial response, to produce a sustained pressor effect (Mosqueda-Garcia et al., 1992). In that study, the cardiovascular responses to ET-1, microinjected into the nTS after BQ-123 were blocked (Mosqueda-Garcia et al., 1992), similar to the results of our study. We therefore conclude that the excitatory gastric motor and hypertensive effects of endothelins in the DVC are mediated through ET₂ receptors.

Because ET-1 has been reported to produce lesions in the rat brain due to long-lasting vasoconstriction (Fuxe et al., 1989b), we were concerned that neurotoxicity might account for the absence of responses to repeated microinjection of ET-1 after BQ-123. The fact that a significant increase in peak intragastric pressure was evoked by L-GLU after BQ-123 is a positive control and indicates that ischemic damage does not account for the absence of the response to a repeat microinjection of ET-1 after BQ-123. Indeed, major lesions involving 30 to 70% of the total volume of the neostriatum were only observed after the injection of ET-1 into this brain region at a dose of 400 pmol (Fuxe et al., 1989a), a dose that is 10-fold higher than the highest dose used in our study and 40 times higher than this one used in our experiments with BQ-123. Additionally, we observed that the cardiovascular effects of ET-1 at a dose of 40 pmol usually lasted 15 to 30 min, whereas the increases in MAP and heart rate as a consequence of the lesions of the nTS lasted for many days (Reis et al., 1977; de Jong et al., 1977). Therefore, the cardiovascular changes in response to ET-1 at a dose of 40 pmol in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRIGP (cmH₂O)</th>
<th>ARIGP (cm²)</th>
<th>PCA (MMI)</th>
<th>GCCA (MMI)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle DVC</td>
<td>0.1 ± 0.1</td>
<td>0.00 ± 0.00</td>
<td>-0.1 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>ET-1 DVC</td>
<td>1.4 ± 0.2*</td>
<td>0.52 ± 0.14*</td>
<td>2.3 ± 0.9</td>
<td>1.3 ± 0.6</td>
<td>29 ± 26</td>
</tr>
<tr>
<td>ET-1 outside DVC</td>
<td>0.1 ± 0.1</td>
<td>0.00 ± 0.00</td>
<td>0.3 ± 1.0</td>
<td>-0.3 ± 0.1</td>
<td>-8 ± 5</td>
</tr>
</tbody>
</table>

* Statistically significant when compared with corresponding mean for vehicle microinjection.

after vagotomy and spinal cord transection are shown in table 3.

**TABLE 2**
The changes in intragastric pressure (PRIGP, peak response; ARIGP, area of the response), pyloric (PCA) and greater curvature (GCCA) contractile activity and MAP induced by microinjection of vehicle and ET-1 (10 pmol) into the DVC and outside the DVC in four animals (means ± S.E.)

Fig. 5. Representative chart recordings from one experiment in which ET-3 (100 pmol) was microinjected into the DVC. Endothelin-3 evoked small increases in gastric motor and decreases in cardiovascular function.

![Graph showing intragastric pressure, pyloric motility, greater curvature motility, heart rate, and mean arterial pressure](image-url)
TABLE 4
Effect of BQ-123 (400 pmol) on L-GLU- and endothelin-evoked in the DVC changes in gastric motor and cardiovascular function

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRIGP (cm H₂O)</th>
<th>ARIGP (cm²)</th>
<th>PCA (MMI)</th>
<th>GCCA (MMI)</th>
<th>HR (bpm)</th>
<th>MAP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.3 ± 0.1</td>
<td>0.00 ± 0.00</td>
<td>0.1 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>ET-1 10 pmol before BQ-123</td>
<td>2.1 ± 0.5a</td>
<td>1.65 ± 0.45a</td>
<td>2.6 ± 0.7a</td>
<td>1.0 ± 0.6</td>
<td>75 ± 38</td>
<td>47 ± 18a</td>
</tr>
<tr>
<td>ET-1 10 pmol after BQ-123</td>
<td>0.3 ± 0.1b</td>
<td>0.10 ± 0.10b</td>
<td>-0.2 ± 0.4b</td>
<td>0.1 ± 0.1</td>
<td>0 ± 0</td>
<td>3 ± 4b</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.0 ± 0.0</td>
<td>0.00 ± 0.00</td>
<td>-0.5 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>ET-3 100 pmol before BQ-123</td>
<td>1.8 ± 0.6a</td>
<td>0.45 ± 0.35</td>
<td>1.5 ± 1.0</td>
<td>0.1 ± 0.0</td>
<td>-17 ± 22</td>
<td>10 ± 8</td>
</tr>
<tr>
<td>ET-3 100 pmol after BQ-123</td>
<td>0.1 ± 0.0b</td>
<td>0.00 ± 0.00</td>
<td>0.3 ± 0.6</td>
<td>-0.3 ± 0.3</td>
<td>3 ± 3</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± S.E. for changes in intragastric pressure (PRIGP), peak response; ARIGP, area of the response), pyloric (PCA) and greater curvature (GCCA) contractile activity as well as heart rate (HR) and MAP for three to five animals.

a Statistically significant when compared with corresponding mean for vehicle microinjection.
b Statistically significant when compared with corresponding mean for ET microinjection.

TABLE 5
Effect of bilateral cervical vagotomy or spinal cord transection (SCT) on changes in intragastric pressure (PRIGP, peak response; ARIGP, area of the response), pyloric (PCA) and greater curvature (GCCA) contractile activity as well as heart rate (HR) and MAP induced by microinjection of ET-1 into the DVC of four to five animals (means ± S.E.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PRIGP (cm H₂O)</th>
<th>ARIGP (cm²)</th>
<th>PCA (MMI)</th>
<th>GCCA (MMI)</th>
<th>HR (bpm)</th>
<th>MAP (mmHg)</th>
</tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>0.1 ± 0.1</td>
<td>0.00 ± 0.00</td>
<td>0.7 ± 0.4</td>
<td>-0.1 ± 0.1</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
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<td>ET-1 10 pmol before vagotomy</td>
<td>2.9 ± 1.0a</td>
<td>2.16 ± 0.42a</td>
<td>0.6 ± 1.0</td>
<td>-0.7 ± 0.8</td>
<td>40 ± 30</td>
<td>21 ± 5a</td>
</tr>
<tr>
<td>ET-1 10 pmol after vagotomy</td>
<td>0.6 ± 0.2b</td>
<td>0.40 ± 0.04b</td>
<td>-0.2 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>6 ± 4</td>
<td>15 ± 9</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.0 ± 0.0</td>
<td>0.00 ± 0.00</td>
<td>-0.5 ± 0.5</td>
<td>0.0 ± 0.2</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>ET-1 40 pmol before SCT</td>
<td>4.3 ± 0.8a</td>
<td>0.9 ± 0.30</td>
<td>5.5 ± 1.5a</td>
<td>2.8 ± 1.3</td>
<td>23 ± 60</td>
<td>20 ± 7a</td>
</tr>
<tr>
<td>ET-1 40 pmol after SCT</td>
<td>1.5 ± 0.2a</td>
<td>0.77 ± 0.54</td>
<td>2.8 ± 0.8</td>
<td>1.0 ± 0.7</td>
<td>-20 ± 11</td>
<td>20 ± 0b</td>
</tr>
</tbody>
</table>

a Statistically significant when compared with corresponding mean for vehicle microinjection.
b Statistically significant when compared with corresponding mean for ET-1 microinjection before vagotomy or SCT.

The potency of ET-3 to affect gastric motor function in the DVC is different from that reported in our previous work (Krowicki and Hornby, 1996). When applied to the dorsal surface of the medulla, ET-3 was equipotent to ET-1 in evoking increases in intragastric pressure and gastric smooth muscle contractile activity. However, 100-fold higher dose of ET-3 than that of ET-1 in the DVC was required to produce changes in intragastric pressure of comparable magnitude to those elicited by ET-1 (Krowicki and Hornby, 1996). We cannot explain these differences; however, the same discrepancy was reported by others (Mosqueda-Garcia et al., 1992) investigating the cardiovascular effects of endothelins in the lower brainstem. When microinjected into the area postrema, both ET isoforms elicited similar cardiovascular effects, whereas their effects in the nTS were opposite (Mosqueda-Garcia et al., 1992). We hypothesize that these differences may be caused by the presence of different ET receptor subtypes in the DVC and area postrema. The fact that ET-3 at the highest dose of 100 pmol was only able to produce increases in peak intragastric pressure comparable to those evoked by ET-1 at a dose of 1 pmol, suggests that ET-3 is affecting ET₃ receptors at this high dose (Rebello et al., 1995). Supportive of this assumption, we were able to abolish the effect of ET-3 on intragastric pressure by preinjection into the DVC of BQ-123, an ET₃ receptor antagonist. It appears that the ET₃ receptor is the function important subtype in the hindbrain, because intracerebroventricular infusion of an ET₃ receptor agonist does not induce discernible c-fos expression in the DVC as opposed to intensive c-fos expression observed after infusion of ET-1 or ET-3 (Zhu and Herbert, 1996).

Because ET-1 is the most potent known vasconstrictor (Yanagisawa et al., 1988), it could be speculated that ET-1, microinjected into the DVC, might act peripherally to increase MAP. This possibility is unlikely, because ET-1, microinjected into the dorsal medulla just lateral to the DVC, does not change MAP (table 2). In addition, because spinal cord transection abolished the hypertensive effect of ET-1, microinjected into the DVC, it is likely that activation of medullary sympathetic outflow is mediating this effect.
We show that microinjection of ET-1 into the DVC increases MAP, whereas ET-3, at the same site, evokes no changes in cardiovascular function (table 1). Endothelin-1, microinjected into the cardiovascular sites of the nTS (the dorsal striop and the commissural subnucleus of the nTS), was reported to decrease heart rate and MAP at doses of 100 to 300 fmol in a volume of 50 to 200 pl (Hashim and Tadepalli, 1992) or 0.5 to 6 pmol in a volume of 60 nl (Mosqueda-Garcia et al., 1992). Endothelin-3 (2–10 pmol in 200 nl) was reported to evoke both tachycardia and hypertension in the nTS (Kuwaki et al., 1990; Mosqueda-Garcia et al., 1992). These differences in cardiovascular responses to endothelins may be because of the different microinjection sites. In our experiments, microinjection of endothelins into the DVC, apparently in the region of the dorsal motor nucleus of the vagus, produced consistent and repeatable gastric motor excitation but no overall significant change in heart rate.

The endogenous source for ET-1 in the DVC is unknown, although ET-1 is widely distributed in the brain of the rat (Yoshimi et al., 1991). A comprehensive morphological mapping study of ET-1 mRNA and immunoreactivity in the human brain (Giaid et al., 1991), revealed that ET-1 mRNA and the peptide are present in cell bodies of the paraventricular hypothalamic nucleus as well as raphe nuclei, which directly project to the DVC and control gastric function (Hornby et al., 1990; Rogers et al., 1980). Nerve terminals with ET-1-like immunoreactivity have been localized in the brainstem medulla (Giaid et al., 1991). However, to our knowledge, no data on the presence of such terminals within the DVC have been reported. Additionally, the presence of ET-1-like immunoreactive cells in the dorsal motor nucleus of the vagus (Giaid et al., 1991) may indicate autocrine or paracrine actions of the peptide.

Another hypothetical source of ET-1 in the DVC is from the peripheral circulation, because the proximity of the DVC to the cerebrospinal fluid bathing the fourth ventricle and its close anatomical association to the area postrema provides likely routes through which circulating agents may reach specific receptors in the DVC. It has been also shown that the nTS may perceive circulating stimuli directly via its own microcirculation in the dorsomedial and lateral commissural subnucleus (Gross et al., 1990). The capillaries in the other subnuclei of the nTS and dorsal motor nucleus of the vagus are considered to have predominantly type II endothelial ultrastructure and are thus presumed to be rather impermeable (Gross, 1992). Accordingly, numerous autoradiographic studies have documented the presence of ET-1 binding sites in the area postrema and medial nTS (Kohzuki et al., 1991; Koseki et al., 1989; van den Buse and Itoh, 1993). This hypothesis is supported by our recent observations that intravenously administered ET-1 evokes vagally-mediated increases in gastric motor function (Krowicki and Hornby, in press).

We hypothesize that our results may somehow contribute to our understanding of the mechanisms leading to gastrointestinal motor and cardiovascular disturbances in hyperinsulinemia. This is because plasma ET-1 levels are elevated in obesity, associated with increased plasma insulin levels (Wolpert et al., 1993; Ferri et al., 1995a) and in insulin-treated diabetic patients (Takahashi et al., 1990; Golfman et al., 1993). Moreover, circulating ET-1 levels increase during insulin infusion in patients with non-insulin dependent diabetes mellitus (Ferri et al., 1995b). Therefore, circulating endothelins may act in the DVC, as well as peripherally, to produce an increase in gastrointestinal motor function and hypertension.

References


Krowicki, Z. K. and Hornby, P. J.: Serotonin and thyrotropin-releasing hormone do not augment their effects on gastric motility on their microinjection.


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