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Short communication

Modulatory effects of endothelin on baroreflex activation in the nucleus of the solitary tract

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Abstract

In this study, we determine the effects of endogenous endothelin on baroreflex activation. After control baroreflex slopes were obtained, the animals received bilateral intra-nucleus tractus solitarii microinjections of saline, or equimolar doses (4 pmol/60 nl) of the endothelin ET_A receptor antagonist cyclo (D-Trp-D-Asp-Pro-Val-Leu (BQ-123), Homopiperinidinyl-CO-Leu-D-Trp(CHO)-D-Trp-OH (BQ-610), or the endothelin ET_B receptor antagonist *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-MeLeu-D-Trp($COOCH_3$)-D-Nle (BQ-788). Intra-nucleus tractus solitarii administration of BQ-123 resulted in a brief initial pressor effect followed by hypotension which resolved by 15 min. The baroreflex slope was significantly enhanced when tested 15 min after BQ-123 treatment (from 2.4 ± 0.5 ms/mmHg to 3.5 ± 0.4 ms/mmHg). Similar effects were observed with the other endothelin ET_A receptor antagonist, except that the hypertensive and hypotensive responses were more pronounced while the baroreflex slope was similarly increased. In contrast, the endothelin ET_B receptor antagonist did not evoke appreciable changes in hemodynamics or in baroreflex slopes. Our results support the concept that endothelin prominently affects reflex cardiovascular function through the endothelin ET_A receptor subtype.

Keywords: Brainstem; Cardiovascular function; Hypertension; Microinjection; (Rat)

1. Introduction

Endothelin is a 21 amino acid polypeptide for which at least two subtypes of receptors (ET_A and ET_B) have been characterized (Yanagisawa and Masaki, 1989). Endothelin may regulate circulatory function by affecting the reflex control of the circulation, in particular, the baroreceptor reflex. Early studies noted that intravenous administration of endothelin-1 evoked exceptionally large bradycardic responses for the corresponding changes in arterial blood pressure (Knuepfer et al., 1989). This was compatible with a sensitization of baroreflex function. However, when endothelin-1 baroreflex responses had been compared to those obtained after the administration of a different pressor agent (phenylephrine), no significant differences have been observed. Furthermore, in different studies, local application of endothelin-1 on the carotid sinus of the dog (Chapleau et al., 1992) or cat (Spyer et al., 1991) was shown to suppress multifiber baroreceptor activity suggesting a direct inhibitory (rather than facilitatory) effect of this peptide on baroreceptor activity.

As endothelin was first discovered and isolated from vascular endothelial cells, initial research efforts did not focus on central nervous system sites of actions. However, subsequent evidence has now indicated that endothelin can exert prominent autonomic effects (Mosqueda-Garcia et al., 1993, 1995), and regulate centrally mediated baroreflex function (Itoh and Van den Buuse, 1991; Lee et al., 1992). Within the brain, endothelin binding sites have been identified in nuclei that participate in baroreflex function, including several areas of the hypothalamus, the rostral ventrolateral medulla, and in the nucleus of the solitary tract (Koseki et al., 1989). In addition, some studies have documented that intracisternal administration of endothelin facilitates the baroreceptor reflex in conscious normotensive (Itoh and Van den Buuse, 1991) and hypertensive rats

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(Van den Buuse and Itoh, 1993). This effect was apparently mediated by changes in parasympathetic tone and proposed to occur at the level of the nucleus tractus solitarii. On the other hand, in different experiments using anesthetized rats, administration of endothelin-1 into the nucleus tractus solitarii resulted in an inhibition of baroreflex sensitivity which was proposed to be secondary to the vascular effects of this peptide (Lee et al., 1992).

The potent vascular effects of endothelin raise the possibility that some of the central cardiovascular actions of this peptide are related to intense vasoconstriction. Although several studies have documented that central administration of other potent vasoconstrictor agents (i.e., vasopressin) do not evoke endothelin-like cardiovascular effects (Mosqueda-Garcia et al., 1995), it has been difficult to dissociate the vascular from the neuronal actions of this peptide. A potential way to circumvent this problem and better document the potential physiological role of endogenous endothelin is with the use of selective receptor antagonists. Specific endothelin receptor antagonists have been described and characterized (Opgenorth, 1995). Some of these agents exhibit high selectivity towards either endothelin ET_A or ET_B receptors. In the present study, with the use of different selective endothelin receptor antagonists, we documented the effects of endothelin receptor blockade on baroreceptor reflex function in the nucleus tractus solitarii of normotensive rats. With this approach, we are able to provide evidence of the type of receptor subtype and the potential physiologic role of endogenous endothelin on the reflex control of the circulation.

2. Methods

2.1. General procedures

Male Sprague–Dawley rats were obtained from Sasco Sprague–Dawley (St. Louis, MO). The animals were housed in an animal room with a light/dark cycle of 12/12 h and were fed rat chow and tap water ad libitum. The animals weighed between 300 and 400 g when used for these experiments. The day of the study, the rats were anesthetized with urethane (1.0 g/kg i.p., plus 300 mg/kg i.v.), and a polyethylene cannula (PE-50) was placed in the femoral vein for administration of drugs. Blood pressure was measured directly through a cannula placed into the femoral artery and connected to a Gould P23ID pressure transducer and a Gould RS3800 polygraph. Heart rate was monitored continuously by a tachograph preamplifier (Gould 13-4615-65) driven by the blood pressure signal.

2.2. Microinjection technique

The animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), with the head flexed downward at an angle of 45°. The dorsal surface of the medulla was exposed by limited craniotomy and the animals rested for at least 30 min before the experiments. For microinjections into the nucleus tractus solitarii, triple-barreled glass microcannulae (1.2-mm o.d., 0.68-mm. i.d., Kwik-Fil, World Precision Instruments, Sarasota, FL) were prepared with an external tip diameter of ~ 40 μm and connected to a nitrogen-pressured, multichannel pneumophoresis pump (World Precision Instruments PV800). One barrel was prefilled with a solution containing glutamate (74 pmol/60 nl), another was prefilled with solutions containing endothelin receptor antagonists such as cyclo(D-Trp-D-Asp-Pro-D-Val-Leu) (BQ-123), Homopiperinidinyl-CO-Leu-D-Trp(CHO)-D-Trp-OH (BQ-610) or *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-MeLeu-D-Trp($COOCH_3$)-D-Nle (BQ-788) and the third one was filled with saline (60 nl) or Fast green ink. Micropipettes were individually calibrated before insertion into the brain tissue and after withdrawal to deliver a volume of 60 nl over 10 s. Diameter of the ejected droplet was measured using a micrometer eyepiece (Acts Instruments, Nashville, TN). Two bilateral microannulae systems were then stereotaxically implanted into the right and left nucleus tractus solitarii using the following anterior–posterior coordinates: 0.0 mm, mediolateral: ± 0.5 mm, vertical: -0.4 mm, using the obex as reference. During the experiment, microinjection of the substance was monitored by observing the movement of the meniscus inside the glass micropipette. Before evaluating the effects of saline or endothelin receptor antagonists on baroreflex function, the injection sites within the nucleus tractus solitarii were selected by defining the most sensitive location that could be found for the effects of glutamate as characterized previously (Mosqueda-Garcia et al., 1993). Glutamate was dissolved in sterile saline to final concentrations in a volume of 60 nl and adjusted to a neutral pH (7.2–7.3) while the endothelin receptor antagonists were initially dissolved in dimethyl sulfoxide (DMSO). Final concentrations (4 pmol/60 nl) of the endothelin receptor antagonists were obtained with subsequent dilutions in sterile saline adjusted to a neutral pH. The final concentration of DMSO was 0.05%. Preliminary unpublished studies in our laboratory had documented no significant effects of control solutions containing this concentration of DMSO.

2.3. Protocol

The animals were allowed to rest for at least 60 min after initial instrumentation. This period was followed by microinjection of 60 nl of saline in all the animals (to assess volume effects). In agreement with previous experiments (Mosqueda-Garcia et al., 1993), microinjection of saline in this small volume had no effect on basal blood pressure or heart rate. This period was followed by testing of the baroreflex responses by assessing the changes in heart rate produced by the pressor action of intravenous bolus administration of phenylephrine (30 μg). This dose

was selected in order to obtain at least increments of 40 mmHg or more in each experimental animal. Points for baroreflex slopes were obtained from recordings made at a fast chart speed (25 mm/s), in which we were able to measure individual intervals between systolic pressure waves and values for systolic and diastolic blood pressure. With this method, we were able to obtain reciprocal correlations between heart rate and systolic blood pressure during basal conditions and during the effects of phenylephrine until they reached the maximum. Typically, we were able to obtain multiple determinations of blood pressure and heart rate intervals including points in which reciprocals changes were observed (linear portion of the curve) and points in which pronounced elevations of blood pressure do not evoke further changes in heart rate intervals (saturation portion of the baroreflex curve). During this control period, two baroreflex slopes were obtained to assess reproducibility of the response. The time interval between these two interventions was not less than 15 min.

The animals were then randomly assigned to one of the following experimental groups: (1) Intra-nucleus tractus solitarii administration of saline (60 nl, control group; $n = 10$); (2) and (3) Intra-nucleus tractus solitarii administration of the endothelin ET_A receptor antagonist BQ123 ($n = 12$) or BQ-610 ($n = 12$), respectively (4 pmol each nucleus tractus solitarii site); or (4) Intra-nucleus tractus solitarii administration of the endothelin ET_B receptor antagonist, BQ-788 ($n = 10$; in equimolar concentrations as the endothelin ET_A receptor antagonists). About 15 min after the microinjection of saline or the specific antagonist into the nucleus tractus solitarii and once all the apparent acute cardiovascular effects of these agents subsided, a new baroreflex response was obtained. The specificity of the antagonists has been previously demonstrated (Mosqueda-Garcia et al., 1993, 1995). Furthermore, in unpublished studies we have documented that microinjection of endothelin ET_A receptor antagonists (i.e., BQ-123) inhibits, in a dose dependent manner, the cardiovascular effects of intra-NTS administration of endothelin (at 2 pmol, 4 pmol, and 2 nmol, 21%, 46%, and 98%, respectively) without affecting those of angiotensin II, vasopressin, neuropeptide Y, clonidine or β-endorphin.

2.4. Data analysis

The reflex bradycardia elicited by the pressor effect of phenylephrine was expressed as heart rate period (in ms) and plotted against the respective increase in systolic blood pressure. Only the increments in blood pressure resulting in reciprocal changes in heart rate (linear portion of the baroreflex curve) were evaluated. The slope of the curve from the linear regression obtained between the increase in pulse period and blood pressure was used to express baroreflex sensitivity. The differences in slope values between the control and the experimental period were compared by paired *t*-test, while the differences between different groups were analyzed by a 2-way analysis of variance followed by Duncan's multiple range test. A P value of < 0.05 was considered to indicate statistical significance.

3. Results

Microinjection of saline into the nucleus tractus solitarii (60 nl each side) did not affect significantly resting blood pressure or heart rate (Table 1). In contrast, bilateral administration of BQ-123 resulted in a biphasic blood pressure response characterized by an initial pressor effect (lasting 8 ± 1.3 min) followed by hypotension which resolved at 12 ± 1.4 min after administration. Similar effects were observed with the other endothelin ET_A receptor antagonist, except that the hypertensive and hypotensive responses were more pronounced. Intra-nucleus tractus solitarii administration of the endothelin ET_B receptor antagonist did not affect significantly blood pressure or heart rate.

In the control or experimental groups, baroreflex slopes during the first and second baseline periods were not significantly different (in the control group: 2.4 ± 0.5 and 2.55 ± 0.4 ms/mmHg, respectively). Microinjection of saline into the nucleus tractus solitarii did not affect the baroreflex slope (2.5 ± 0.4). In the group pretreated with intra-nucleus tractus solitarii administration of BQ-123 (4 pmol per site), the baroreflex slope was significantly enhanced when compared to pre-drug slopes (Fig. 1, upper tracings). Similar enhancement of baroreflex slope was observed after the administration of the other endothelin ET_A antagonist, BQ-610; baroreflex slope increase from a baseline of 2.3 ± 0.4 ms/mmHg to 3.16 ± 0.8 ms/mmHg ($P > 0.05$). In contrast, microinjection of the endothelin ET_B receptor antagonist did not evoke appreciable changes

Table 1
Changes in systolic blood pressure after administration of saline or different endothelin antagonists into the nucleus tractus solitarii of anesthetized rats

SBP	CON (mmHg)	SAL (mmHg)	CON (mmHg)	BQ-123 (mmHg)	CON (mmHg)	BQ-610 (mmHg)	CON (mmHg)	BQ-788 (mmHg)
0–5	125 ± 6	123 ± 4	115 ± 6	125 ± 5[a]	121 ± 5	159 ± 4[a]	129 ± 4	133 ± 8
5–10		124 ± 4		100 ± 8[a]		111 ± 4[a]		136 ± 6

The maximal changes in systolic blood pressure (SBP) were evaluated at 0 to 5 min and 5 to 10 min after bilateral intra-nucleus tractus solitarii administration of saline (SAL; 60 nl), the endothelin ET_A antagonists BQ-123 and BQ-610 and the endothelin ET_B receptor antagonist BQ-788.
[a] Indicates significant difference from respective control period $P < 0.05$.

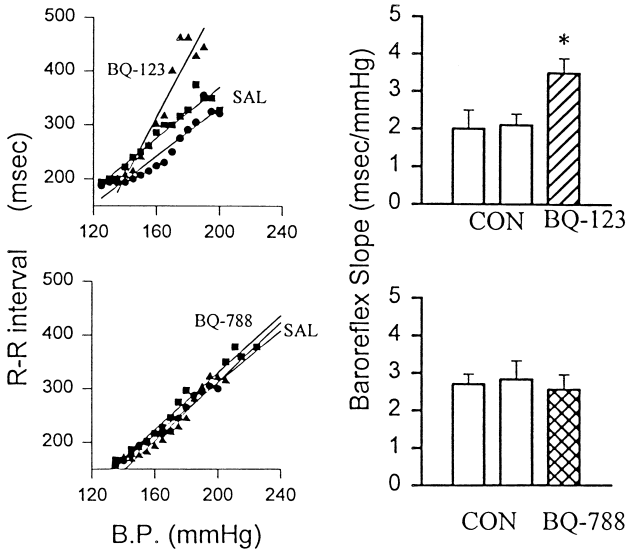

Fig. 1. Effects of endothelin receptor antagonists on baroreflex function in urethane-anesthetized normotensive rats. The graphs to the left present the baroreflex slopes obtained before (control 1 = circles and control 2 = squares) or after microinjection of the endothelin ET_A receptor antagonist BQ-123 (upper graph, fill triangles) or the endothelin ET_B receptor antagonist BQ-788 (bottom graph, filled triangles) in two different animals. The graphs to the right present the mean baroreflex slopes during the two control periods (CON, empty bars) or after the administration of BQ-123 (hatched bar, upper graph) or after BQ-788 (cross-hatched bar, lower right graph). * Indicates significant difference ($P < 0.05$) from control periods. Vertical lines in the middle of the bars represent S.E.M.

in the baroreflex slope when compared to the control period (Fig. 1, bottom tracings).

4. Discussion

In the present study, we observed an increased gain of the baroreflex slope after the animals were pretreated with intra-nucleus tractus solitarii administration of endothelin ET_A receptor antagonists but not after endothelin ET_B antagonists or saline. The effects on the endothelin ET_A receptor antagonists support an important modulatory role of an endothelin-like peptide on baroreflex activation. In particular, our findings indicate an inhibitory effect of endothelin on baroreflex function in the nucleus tractus solitarii which seems to be mediated by the endothelin ET_A receptor subtype. Some other studies have documented endothelin actions on baroreflex activity. Results of these have been controversial since an inhibitory (Lee et al., 1992) or facilitatory (Itoh and Van den Buuse, 1991) actions have been demonstrated. Although factors such as dose, route of administration, and state of consciousness of the animal may account for these discrepancies, it is the potent intrinsic vasoconsrictive properties of endothelin which limits the interpretation and physiological relevance of results obtained in many pharmacological studies. Our present approach circumvents this problem by inferring endothelin actions with the use of specific receptor antagonist. The selectivity of this response was documented by using two different endothelin ET_A receptor antagonists, while the specificity was demonstrated by the lack of effect of the endothelin ET_B receptor antagonist. Since these antagonists lack intrinsic vasoconstrictive properties (Opgenorth, 1995; Mosqueda-Garcia et al., 1995) it is unlikely that the effects on baroreflex activation relate to secondary vascular effects.

We decided to study baroreflex function with rapid administration of a single bolus dose of a pressor agent. We selected this method because it allowed us to evaluate in a fast and accurate way the baroreflex slope in relative short time-interval, in the same experimental animal, and under control and experimental conditions. This method is perhaps the most appropriate when evaluating the effects on baroreflex function of substances with a short half-life or in situations in which the drug effect is not stable over time (i.e., variation of the level of anesthesia and/or physiological conditions of the animal). One potential limitation is that we were unable to determine the threshold portion of the baroreflex slope and consequently, define the entire sigmoid baroreflex curve. The advantage of obtaining the entire baroreflex curve is that someone can be assured that the slope falls in the operating range of the curve and that the changes in pressure will transverse the linear portion of the blood pressure/R–R interval relationship. On the other hand, to our knowledge there is no evidence that in normotensive rats the operating range of baroreflex function is altered and that the use of bolus administration of a pressor agent method yields opposite or highly dissimilar results from other methods studying baroreflex activity.

At this time, it is unclear which of the endogenous isoforms of endothelin is associated with inhibition of baroreflex activation. While the hypertensive and tachycardia response evoked by intra-nucleus tractus solitarii administration of endothelin-3 is compatible with baroreflex inhibition (Mosqueda-Garcia et al., 1993), the facilitation of baroreflex function after endothelin ET_A receptor antagonists would suggest the participation of an endothelin-1-like peptide. In any event, the fact that microinjection of the endothelin ET_A receptor antagonists affected per se resting blood pressure strongly suggests that in the nucleus tractus solitarii a tonic release of an endothelin-like peptide can affect reflex cardiovascular function. Furthermore, the inhibitory effects of endothelin ET_A receptor activation on the baroreflex are in agreement with a potential hypertensinogenic role of this family of peptides. First, the prolonged vasoconstriction produced by endothelin may result in baroreflex resetting and reduced response which is characteristic of hypertensive conditions. Second, at the carotid sinus level, endothelin can reduce baroreceptor discharge which may result in unopposed hypertension (Chapleau et al., 1992). Finally, in the nucleus tractus

solitarii, the inhibitory actions of endothelin ET_A receptor activation evoked by neuronally-produced endothelin may result in heightened sympathetic outflow which can result or aggravate high blood pressure levels.

In summary, our results support the concept that endothelin prominently affects reflex cardiovascular function and this may contribute to the overall hypertensinogenic actions of this family of peptides.

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