Calcitonin Gene-Related Peptide Terminates Long-Lasting Vasopressor Responses to Endothelin 1 In Vivo

Merlijn J.P.M.T. Meens, Nadine J.A. Mattheij, Jelly Nelissen, Pieter Lemkens, Matthijs G. Compeer, Ben J.A. Janssen, Jo G.R. De Mey

Abstract—Slow dissociation of endothelin 1 from its endothelin A receptors is responsible for the long-lasting vasoconstrictor effects of the peptide. We showed recently that calcitonin gene-related peptide selectively terminates long-lasting contractile responses to endothelin 1 in isolated rat mesenteric arteries. Here we assessed whether the antiendothelinergic effect of calcitonin gene-related peptide is vascular bed specific and may terminate long-lasting pressor responses to exogenous and locally produced endothelin 1 in vivo. Regional heterogeneity of the calcitonin gene-related peptide/endothelin A receptor cross-talk was explored in arteries isolated from various rat organs. Endothelin A receptor-mediated arterial contractions were terminated by calcitonin gene-related peptide in rat mesenteric, renal, and spermatic arteries but not in basilar, coronary, epigastric, gastric, splenic, and saphenous arteries. Endothelin A receptor antagonism only ended endothelin 1–induced contractions in spermatic arteries. In anesthetized rats, instrumented with Doppler flow probes to record regional blood flows, long-lasting pressor and vasoconstrictor responses to an intravenous bolus injection of endothelin 1 or big endothelin 1 were transiently reduced by sodium nitroprusside (NO donor) but terminated by intravenously administered calcitonin gene-related peptide. In conscious rats, calcitonin gene-related peptide but not sodium nitroprusside terminated prolonged (>60-minute) pressor responses to endothelin 1 but not those to intravenous infusion of phenylephrine. In conclusion, pressor responses to circulating and locally produced endothelin 1 that are resistant to endothelin receptor antagonism and NO can be terminated by a regionally selective effect of calcitonin gene-related peptide. Calcitonin gene related peptide receptor agonism may represent a novel strategy to treat endothelin 1–associated cardiovascular pathologies. (Hypertension. 2011;58:99-106.) ● Online Data Supplement

Key Words: endothelin 1 ■ CGRP ■ vasculature ■ ET\textsubscript{A} ■ vascular resistance

Endothelin (ET) 1 (ET-1), one of the most potent vasoconstrictors, is present throughout the cardiovascular system.\textsuperscript{1-3} It activates endothelin A (ET\textsubscript{A}) and endothelin B (ET\textsubscript{B}) receptors. ET\textsubscript{A}-induced effects, such as long-lasting vasoconstriction, are considered deleterious. ET\textsubscript{B}-mediated effects, such as vasodilatation and scavenging of ET-1, may counterbalance harmful ET\textsubscript{A}-mediated events.\textsuperscript{4-7} However, recent studies have shown adverse ET\textsubscript{B}-mediated signaling as well.\textsuperscript{8-10} Because ET-1–induced effects contribute to cardiovascular diseases,\textsuperscript{2-11} cancers,\textsuperscript{8} and pain,\textsuperscript{10} ET receptor antagonists have been developed to inhibit harmful effects of the peptide.\textsuperscript{12-15} These drugs have favorable effects in animal studies, but clinical trials with ET receptor antagonists have not been as successful as anticipated.\textsuperscript{16,17} We proposed recently that this may be because of the atypical molecular pharmacology of ET-1 (for review see Reference \textsuperscript{3}). The binding of ET-1 to ET\textsubscript{A} is polyvalent (ie, ET-1 has ≥2 binding sites on ET\textsubscript{A}), “tight” (ie, ET-1 dissociates very slowly from ET\textsubscript{A}), and refractory to antagonists (ie, antagonists cannot fully reverse ET\textsubscript{A}-mediated signaling).\textsuperscript{18} This peculiar pharmacology of ET-1 is responsible for the long-lasting pressor responses that can be observed after intravenous administration of the peptide in rodents and humans.\textsuperscript{1,19} These pressor effects of ET-1 may trigger counterregulatory mechanisms needed to maintain adequate blood pressure regulation. For instance, endothelium-derived NO can reduce ET-1–induced vasoconstrictions\textsuperscript{20} and inhibits the production of ET-1.\textsuperscript{21} In addition, vasodilators like calcitonin gene-related peptide (CGRP), NO, and substance P released from sensory-motor nerves (SMNs) can reduce ET-1–induced vasoconstrictions, and endogenously released CGRP can prevent ET-1–induced increases in blood pressure.\textsuperscript{22}

Established complexes between ET-1 and ET\textsubscript{A} have a half-life of ≤77 hours.\textsuperscript{2} Because of this tight binding of ET-1 on ET\textsubscript{A}, functional antagonism by vasodilators like NO can transiently reduce but not terminate ET\textsubscript{A}-mediated vasoconstrictions.\textsuperscript{18} Moreover, ET-1/ET\textsubscript{A} complexes are not dissociated by ET receptor antagonists, and this greatly reduces the capacity of ET receptor antagonists to inhibit ET\textsubscript{A}-mediated signaling.\textsuperscript{18,23,24} These observations imply that compounds
that accelerate dissociation of ET-1/ET_A complexes might be more efficacious at terminating ET_A-mediated signaling and treating ET-1 related diseases. We have shown that the endogenously released vasodilator CGRP is more potent during ET-1–induced contractions than during phenylephrine (PHE)-induced contractions. This suggests that CGRP acts as more than a functional antagonist during contractions induced by ET-1. More recently, we reported that endogenously released CGRP terminates contractile effects of ET-1 in rat mesenteric arteries by promoting dissociation of pre-existing ET-1/ET_A complexes.

Here, we tested the hypothesis that CGRP/ET_A receptor cross-talk in several vascular beds limits the long-lasting pressor effects of ET-1 in vivo. We used arteries isolated from different vascular beds of the rat and evaluated effects of ET receptor antagonism and CGRP during established ET-1–induced contractions and anesthetized and conscious Wistar Kyoto rats in which we assessed effects of ET-1, big-ET-1, CGRP, and NO on local blood flows, mean arterial pressure (MAP), and heart rate (HR). The resistance arteries and vascular beds that were examined were selected on the basis of their contribution to blood pressure regulation and possible involvement in ET-1–related pathophysiology. We report that CGRP terminates long-lasting ET-1–induced vasopressor effects in several vascular beds not only in vitro but also in vivo where it culminates in blood pressure lowering. Our findings suggest that CGRP receptor activation may be considered for the treatment of diseases involving ET-1.

Methods

Experimental protocols were conducted according to international guidelines (American Physiological Society Guiding Principles for the Care and Use of Vertebrate Animals in Research and Training) and were approved by the Maastricht University Ethics Committee on Experimental Animal Welfare.

Organ Chamber Studies

Male Wistar Kyoto rats, 16 weeks old (Charles River, Maastricht), were euthanized by CO2 inhalation. Mesenteric (second-order side branches of the superior mesenteric artery), basilar, coronary septal, epigastric, gastric, renal arcuate, saphenous, spermatic, and splenic arteries were isolated and were directly mounted in a wire myograph. Before the experiment, arterial segments (2 mm long) were distended to a diameter corresponding with 90% of the passive diameter at a transmural pressure of 100 mm Hg (0.9 D100).

To address regional heterogeneity of vasorelaxing responses to CGRP and CGRP/ET_A receptor cross-talk at the level of resistance arteries, we recorded arterial responses to capsaicin (CAPS), CGRP, ET-1, and sarafotoxin 6c. Details are given as Supplementary Methods in the online Data Supplement (please see http://hyper.ahajournals.org).

In Vivo Experiments

Male Wistar Kyoto rats, 16 weeks old, were housed in groups with free access to food and water. After instrumentation with chronic catheters, rats were housed individually.

To determine whether the CGRP/ET_A receptor cross-talk is vascular bed specific, we used anesthetized rats fitted with heparinized indwelling polyethylene catheters and monitored effects of pharmacological interventions on local blood flows and MAP. Details are given as Supplementary Methods in the online Data Supplement.

To assess whether CGRP/ET_A receptor cross-talk occurs in vivo when all of the cardiovascular reflex control mechanisms are functional, we determined hemodynamic parameters in conscious, unrestrained rats fitted with heparinized indwelling polyethylene catheters and assessed the effect of pharmacological interventions on MAP. Details are given as Supplementary Methods in the online Data Supplement.

Drugs and Solutions

Krebs Ringer bicarbonate-buffered physiological salt solution (KRB) was prepared as described previously. High K⁺-KRB solution was KRB in which all of the NaCl was replaced by KCl. Solutions containing 40 mmol/L of K⁺ were prepared by mixing KRB and K⁺-KRB. Pharmacological interventions were obtained from Sigma Aldrich or Bachem and were diluted in KRB solution, ethanol, or dimethyl sulfoxide. Details are given as Supplementary Methods in the online Data Supplement.

Statistics

Contractile responses are expressed as percentage of K⁺_max. Relaxing responses are expressed as percentage of the level of precontraction. Concentration-response curves were fitted to a nonlinear sigmoid regression curve (GraphPad Prism 5.0) to determine EC50 values (ie, the concentration of the agonist required for 50% of the maximal response) and maximal responses. Changes in MAP, HR, and vascular resistances are expressed relative to stable baseline values recorded during 15 minutes before a pharmacological intervention. All of the data are shown as mean±SEM. Statistical significance of differences was assessed using either 1-way ANOVA or 2-way ANOVA. Bonferroni post hoc test was used to compare multiple groups. A P value <0.05 was considered statistically significant.

Results

To study regional heterogeneity of arterial responses in vitro we used arteries from 9 vascular beds. The diameters of these vessels were comparable and all of the arteries contracted in response to 40 mmol/L of K⁺ (Table S1, available in the online Data Supplement). Figure 1 summarizes their responses to endogenously released neuropeptides and to exogenous CGRP. In mesenteric resistance arteries contracted with 40 mmol/L of K⁺, a high concentration of CAPS (1 µmol/L), an agonist of transient receptor potential cation channel subfamily V member 1 (TRPV1), caused an initial transient relaxation followed by a subsequent increase of the contractile amplitude (Figure 1A and 1B). We previously attributed this to stimulation and subsequent desensitization of peri-arterial SMN-releasing CGRP. This response to CAPS differed markedly between different types of artery (Figure 1B). The response was most marked in mesenteric arteries and intermediate in, for instance, coronary septal and renal arteries, and was not observed in spermatic and basilar arteries. Maximal relaxing responses of depolarized arteries to exogenous CGRP (100 nmol/L) also differed markedly between types of arteries (Figure 1C), and this regionality differs from that observed with CAPS. For instance, in intrarenal arteries, a much larger response to CGRP was observed than to CAPS. These findings confirm that the density of peri-vascular SMN differs between vascular beds and indicate that the relaxing responses to CGRP do not correlate with the presence of these nerves. Furthermore, in specific cases, peri-arterial SMN may lack functional TRPV1 (eg, spermatic arteries), or these channels may be less prone to desensitization by CAPS (eg, basilar arteries).

In rat mesenteric arteries, CGRP is more efficacious at reversing ET-1–induced contractions than not only other
vasodilators but also ET receptor antagonists. This was verified in arteries isolated from a variety of vascular beds. All of the types of artery contracted in response to ET-1 (1 to 32 nmol/L; Table S1), but no type of artery contracted in response to the ET\textsubscript{A} agonist sarafotoxin 6c (1 to 128 nmol/L; Figure S1). The sensitivity to ET-1 was in the nanomolar range in all types of arteries, except for gastric arteries, where it was lower, and the maximal effect of ET-1 was larger than that of 40 mmol/L of K\textsuperscript{+} (Table S1). Established arterial responses to ET-1 were not modified by the ET\textsubscript{A} antagonist BQ788 (1 μmol/L; data not shown). In most arteries, they were little reduced by the ET\textsubscript{A} antagonist BQ123 (1 μmol/L; Figure 2) and well maintained when free agonist and antagonist were removed (Figure 2) but could persistently be terminated by exogenous CGRP (100 nmol/L; Figure 2). The former 2 observations were made in all types of arteries except for spermatic arteries (Figure 2). The latter was most marked in mesenteric, renal, and spermatic arteries (Figure 2C through 2F); intermediate in epigastric and splenic arteries (Figure 2); and not observed in basilar, coronary, gastric, and saphenous arteries (Figure 2). This indicates that an antiendothelinergic effect of CGRP can be observed in several but not all types of arteries.

To assess whether the CGRP/ET\textsubscript{A} receptor cross-talk is sufficiently widespread along the systemic arterial tree to affect local and/or total R and MAP, we used anesthetized rats and evaluated the effects of ET-1, CGRP, and SNP on MAP, HR, and R in the mesenteric (MR), renal (RR), and hindquarter (HQR) vascular beds. As shown in Figure 3, ET-1 (1 μg/kg, bolus IV) caused long-lasting increases of the RR (Figure 3C), MR (Figure 3D), and HQR (Figure 3E). This resulted in a small but significant (\(P<0.05\)) long-lasting increase in MAP (Figure 3F), which was not attributed to an increased HR (data not shown). During established ET-1–induced pressor responses, SNP (30 μg/kg, bolus IV) tended to decrease RR (Figure 3C) and significantly (\(P<0.05\)) decreased MR (Figure 3D), HQR (Figure 3E), and MAP (Figure 3F). The effects of SNP were transient and were fully reversed within 8 minutes. In contrast, depressor responses to CGRP (3 μg/kg, bolus IV) reversed the ET-1–induced rise of RR (Figure 3C), MR (Figure 3D), and HQR (Figure 3E) and the increase in MAP (Figure 3F).

Because ET-1 is a paracrine factor rather than a circulating hormone, we assessed the effect of IV-administered big-ET-1 (1 μg/kg, bolus IV; Figure 4). Big-ET-1 did not increase HR (data not shown) but increased RR (Figure 4C), MR (Figure 4D), and HQR (Figure 4E). Consequently, big-ET-1 caused significant (\(P<0.05\)) and long-lasting (>60-minutes) increases in MAP (Figure 4F). During established big-ET-1–induced pressor effects, SNP (30 μg/kg, bolus IV) decreased RR (Figure 4C), MR (Figure 4D), and HQR (Figure 4E). This resulted in prominent depressor responses (Figure 4F). However, similar to the effects of SNP that we observed during established ET-1–induced pressor responses, the effects of the NO donor during big-ET-1–induced effects were transient and were reversed rapidly. CGRP administration (3 μg/kg, bolus IV) during established big-ET-1–induced increases in MAP terminated the increase in RR (Figure 4C), MR (Figure 4D), and HQR (Figure 4E) and caused a large depressor response (Figure 4F).

To assess whether CGRP also terminates ET-1–induced effects in vivo when baroreflex control mechanisms are not affected by anesthesia, we used conscious unrestrained rats. Both ET-1 (2.2 μg/kg, infused IV during a 7-minute period) and PHE (continuous IV infusion, 7 μg/kg per minute) caused long-lasting, stable pressor responses of equal amplitude (ET-1 133±8% and PHE 139±5% of basal MAP, respectively; Figure 5B and 5E). The vasopressor effects of PHE or ET-1 were accompanied by minor bradycardia (Figure 5C and 5F). CGRP (3 μg/kg, bolus IV)–induced depressor responses terminated ET-1–induced pressor responses (Figure 5D and 5E). This was not the case for depressor responses induced by SNP, which, again, were only transient (30 μg/kg, bolus IV; data not shown). Depressor responses induced by CGRP during PHE infusions were reversed within 20 minutes (Figure 5A and 5B), whereas this
was not the case for CGRP-induced depressor responses during long-lasting ET-1–induced pressor responses (Figure 5D and 5E).

**Discussion**

The main findings of this work are as follows: (1) CGRP terminates long-lasting contractile effects of ET-1 in several types of arteries; (2) in vivo this effect terminates increases in local vascular resistance and blood pressure in response to exogenous ET-1; (3) this mechanism can terminate long-lasting vasopressor effects of locally produced ET-1; and (4) CGRP-ET-1 interactions can be demonstrated in conscious unrestrained rats.

ET-1 is involved in several diseases.2,8,10,11,16 The molecular pharmacology of ET-1 is peculiar; it binds tightly to ET receptor antagonism but can be permanently reduced by calci-тонin gene-related peptide (CGRP) in several types of arteries. In basilar (A), coronary (B), epigastric (C), gastric (D), mesenteric (E), renal (F), saphenous (G), spermatic (H), and splenic (I) arteries, 32 nmol/L of ET-1–induced contractions are of equal amplitude between the time control and experimental groups of arteries (a). At b, arteries are exposed to either (i) nothing (time control, white bars) or (ii) CGRP (100 nmol/L; black bars) or BQ123 (1 μmol/L; red bars). CGRP relaxed epigastric, mesenteric, renal, saphenous, spermatic, and splenic arteries. BQ123 relaxed saphenous and spermatic arteries. After 10 minutes, BQ123 or CGRP was removed from the organ chambers, and wall tension was monitored 10 minutes after removal of the compounds (c). In case of mesenteric, renal, and spermatic arteries, transient presence of CGRP caused permanent relaxation of the ET-1–induced contractions (black bars). In the case of saphenous and spermatic arteries, the ET-1–induced contractions were permanently reduced by transient exposure to BQ123 (red bars). Data are expressed as the percentage of K+/max and are shown as mean ± SEM (n = 6 to 7). **P < 0.01, *P < 0.05. **

Figure 5D and 5E).

unbound agonist.3,18 In addition, tight binding of ET-1 to ET receptor antagonists have been developed to inhibit ET-1–mediated signaling.12–15 However, these compounds cannot reverse established ET-1/ETA complexes and are, thus, less effective (or even ineffective; this study) in reversing than preventing ET-1–induced effects in vitro and in vivo.16–18,33

We recently evaluated candidate endogenous vasodilators for selective ET-1 counterbalancing effects.18 In rat mesen-teric resistance arteries, endothelium-derived vasodilators acted as functional antagonists of ET-1–induced effects. In contrast, stimuli of SMN and exogenous CGRP promoted dissociation of established ET-1/ETA complexes and could therefore terminate contractile effects of ET-1.18 The effects of SMN stimulation and of exogenous CGRP were inhibited by the CGRP receptor antagonists CGRP8–37 and BIBN4096BS, indicating that they were mediated by CGRP receptor activation.18 In this study we evaluated whether the interaction between CGRP receptor activation and ET recep-
tor function is a local phenomenon or is sufficiently widespread to result in systemic effects.

In resistance arteries isolated from 9 vascular beds, ET-1 but not sarafotoxin 6c caused contractions. The potency of ET-1 was comparable in all of the arteries except in gastric arteries, where it was lower. In line with earlier findings indicating that endothelin receptor antagonists can prevent but not reverse binding of ET-1 to its receptors,18,23,24 established ET-1–induced contractions were not modified by ETB antagonism. ETA antagonism reduced ET-1–induced signaling in mesenteric resistance arteries only. In addition, all of the contractions to ET-1 persisted after removal of free agonist and antagonist. These observations are all in line with long-lasting activation of ETA receptors because of slow dissociation of their natural ligand from its receptors3,18 The effect of exogenous CGRP was determined during the ETA-induced contractions. Marked and persistent relaxing effects of CGRP were observed in 3 of the 9 types of arteries. In basilar, coronary, and gastric arteries, no significant effect was observed. Thus, unlike effects of ET-1, which caused contractions in all of the arteries, effects of CGRP display regional heterogeneity. This may mean that, in the pathophysiology of coronary and cerebral vascular disease, the harmful role of ET-1 cannot be tempered by CGRP. Dedicated analyses of the coronary and cerebral circulations will be required to establish this in more detail. Relaxing effects of CGRP during partial depolarization displayed similar regional heterogeneity as the antiendothelinergic effect of the neuropeptide. Therefore, we suggest that regional differences in the expression of the subunits of the heterotrimeric CGRP receptor34 contribute to the heterogeneity.

CGRP is a neurotransmitter rather than a circulating hormone.29,35 Therefore, we assessed whether effects of exogenous CGRP coincide with effects of SMN stimulation. Exogenous CGRP relaxed several arteries, for example, basilar and spermatic arteries, which did not relax in response to CAPS. Thus, CGRP may have paracrine or endocrine functions (eg, regulation of blood flow distant from its source), and CGRP receptor agonism can terminate ET-1–induced effects irrespective of the density of sensory-motor

Figure 3. Calcitonin gene-related peptide (CGRP), but not sodium nitroprusside (SNP), terminates endothelin (ET) 1–induced vaso-pressor responses in anesthetized rats. Animals received ET-1 (1 μg/kg IV) only (control; black), ET-1 (1 μg/kg IV) followed by SNP (30 μg/kg IV; blue), or ET-1 (1 μg/kg) followed by CGRP (3 μg/kg IV; red). A and B, Typical tracings displaying systolic (yellow), diastolic (light red), and mean arterial pressure (MAP; dark red) during experiments in which rats received SNP (A) or CGRP (B) during ET-1–induced vasopressor responses. From such tracings, data were collected at points a, b, c, and d. Averages were calculated and are depicted in C through F. C through F, Renal vascular resistance (RR), mesenteric vascular resistance (MR), hindquarters vascular resistance (HQR), and mean arterial pressure (MAP), respectively. Data are expressed as percentage of basal RR or basal MAP and are shown as mean±SEM (n=6). *P<0.05 vs basal RR or basal MAP.
innervation. It has been reported that CGRP can act as an endothelium-derived relaxing factor in at least some vascular beds.\textsuperscript{29,35} However, this remains to be explored in more detail.

Given the observations in arteries from 2 major vascular beds (mesenteric and renal vascular beds), we assessed whether CGRP is more efficacious at reversing ET-1–induced vasopressor responses compared with NO in vivo. As noted before,\textsuperscript{1,36} IV bolus injection of ET-1 resulted in a long-lasting increase in MAP after a transient depressor effect. This pressor response was at least partly caused by increased MR, RR, and HQR. Subsequent bolus IV administration of SNP only transiently reduced MAP, MR, and HQR, confirming that NO causes transient vasodilatation during ETA-mediated signaling (functional antagonism versus ETA).\textsuperscript{18} In contrast, IV bolus administration of CGRP during the long-lasting pressor response to ET-1 caused a long-lasting drop in HQR and normalization of RR and MR, which resulted in a long-lasting drop in MAP. Discrepancies between the time courses of the effects of SNP and CGRP may be attributed to differences in circulating half-lives\textsuperscript{37,38} or in the mechanism of action of both compounds. Because ET-1 is a locally produced paracrine mediator rather than a circulating hormone, we performed similar experiments with big-ET-1. Big-ET-1 is inactive by itself and acts as a substrate for ET-1 production within the arterial wall.\textsuperscript{39} Long-lasting pressor and vasoconstrictor responses to IV big-ET-1 were again markedly but only transiently reduced by SNP. In contrast, IV bolus administration of CGRP resulted in a normalization of MAP and arterial resistances. In conclusion, ET-1–induced effects initiated by either exogenous or locally produced ET-1 can be reversed by exogenously administered CGRP. Whether CGRP receptor activation by endogenous CGRP can also reverse effects induced by endogenously released ET-1 remains to be established.

Finally, in an attempt to discriminate between pharmacokinetic and pharmacodynamic mechanisms underlying our observations in vivo, we used conscious unrestrained rats receiving either short-term administration of ET-1 or continuous infusion of PHE. This also allowed evaluation of the
intensity of the CGRP effect in conditions of intact baroreflex control. During PHE-induced pressor responses, CGRP-induced depressor responses. In line with the 7-minute plasma half-life of CGRP, the responses were fully reversed within 20 minutes. In contrast, during pressor responses initiated by short-term application of ET-1, CGRP-induced depressor responses were long lasting (>55 minutes) and permanently reduced ET-1-induced pressor responses. These observations indicate that, in line with earlier in vitro findings, the effects of CGRP receptor activation are fundamentally different depending on the pressor-inducing stimulus. This could involve cAMP/adenyl cyclase–independent indirect allosteric modulation of ET-1/ET\textsubscript{A} complexes by CGRP similar to our in vitro findings. However, whether CGRP is able to promote dissociation of established ET-1/ET\textsubscript{A} complexes in vivo remains to be directly demonstrated.

Perspectives
Our results demonstrate that the endogenous vasodilator neuropeptide CGRP is more efficacious than ET receptor antagonism and other relaxing agents in reversing contractile response to ET-1 in several types of resistance arteries. In anesthetized and conscious rats, this effect is sufficiently widespread to terminate increases in local R and blood pressure in response to exogenously administered and endogenously synthesized ET-1. We, therefore, propose that CGRP receptor activation may be considered for treatment of diseases that involve ET-1.

Figure 5. In conscious rats, calcitonin gene-related peptide (CGRP) terminates ET-1–induced pressor responses. A, Typical tracing displaying 10-second averages of mean arterial pressure (MAP) illustrating transient CGRP-induced (3 µg/kg iv) depressor responses during pressor responses induced by continuous phenylephrine (PHE) infusion (7 µg/kg/min iv). B, Effects of continuous PHE infusion (black) and an IV CGRP bolus injection (black arrow) during continuous PHE infusion (red) on MAP. C, Effects of continuous PHE infusion (black) and an IV CGRP bolus injection (black arrow) during continuous PHE infusion (red) on heart rate (HR). D, Typical tracing displaying 10-second averages of MAP illustrating termination of lasting ET-1–induced (2.2 µg/kg iv infusion during 7 minutes) pressor responses by an IV CGRP (3 µg/kg iv) bolus injection. E, Effects of short-term ET-1 infusion (black) and an IV CGRP bolus injection (black arrow) during the following pressor responses (red) on MAP. F, Effects of short-term ET-1 infusion (black) and an IV CGRP bolus injection (black arrow) during the following pressor responses (red) on HR. Data are expressed as the percentage of basal MAP or basal HR and are shown as mean±SEM (n=7 to 9). *P<0.05 vs basal control values. †P<0.05 control vs experimental group. T=0 equals the start of either transient ET-1 infusion or continuous PHE infusion.

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Disclosures
None.

References


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CGRP TERMINATES LONG-LASTING VASOPRESSOR RESPONSES TO ENDOTHELIN-1 IN VIVO

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Short title: CGRP terminates ET-1

Supplementary methods

Organ chamber studies.

After stretching, arterial segments were contracted with 40 mM K+ (K+max) and were exposed to CAPS (1 μM, 20 min (Fig 1)). This served to monitor presence of vasodilator peri-arterial SMN and to persistently desensitize these structures that can be acted upon by ET-1. Subsequently, arteries were contracted with 40 mM K+ and relaxing responses to exogenous CGRP (100 nM) were determined. Thereafter, a concentration-response curve (CRC) for ET-1 (0.25 – 32 nM) was generated and the effect of BQ123 (selective ET_A antagonist; 1 μM) or BQ788 (selective ET_B antagonist; 1 μM) was determined. Next, both unbound ET-1 and the ET receptor antagonist(s) were removed from the organ chambers and wall tension was monitored for another 8 min. In another set of experiments arteries were exposed to ET-1 (32 nM) after desensitization of their SMN. After 10 min of exposure to ET-1, the peptide was removed from the organ chambers and the effect of transient (10 min.) exposure to CGRP (100 nM) or BQ123 (1μM) was assessed. In yet another set of experiments arteries were exposed to sarafotoxin 6c (1 – 128 nM) after desensitization of their SMN.

In vivo experiments.

Regional effects of the CGRP/ET_A receptor crosstalk were assessed in anesthetized (pentobarbital; 50 mg/kg) rats. During anesthesia, heparinized (5 U/ml) indwelling polyethylene catheters were introduced in the femoral artery and femoral vein. The arterial catheter was connected to a pressure transducer (Micro Switch 150 PC). In addition, regional blood velocity through the renal artery, superior mesenteric artery and the abdominal aorta was measured using Doppler flow probes. Resistances (R) of the Renal (RR), mesenteric (MR) and hindquarter vascular (HQR) vascular beds were calculated as MAP divided by mean flow. Pharmacological interventions (CGRP (3 μg/kg), big-ET-1 (1 μg/kg), ET-1 (1 μg/kg) or sodium nitroprusside (SNP; 30 μg/kg), all dissolved in 100 μL 0.9% NaCl) were administered via the catheter in the femoral vein (See Fig. 3 and Fig. 4).

To assess whether CGRP/ET_A receptor crosstalk occurs in vivo when all cardiovascular reflex control mechanisms are functional, we determined hemodynamic parameters in conscious, unrestrained rats. Heparinized (5 U/ml) indwelling polyethylene catheters were introduced in the
femoral artery and femoral vein two days before the experiments. At the day of the experiment, the arterial line was connected to a pressure transducer (Micro Switch 150 PC) and the signal was sampled at 2.5 kHz. MAP and HR were derived from this signal using the IDEEQ data-acquisition system. The venous line was extended outside the cage and filled with 0.9% NaCl. Hemodynamics were allowed to stabilize before the pharmacological interventions. The following compounds were tested: CGRP (3 μg/kg, iv bolus dissolved in 100µL 0.9% NaCl), ET-1 (2.2 μg/kg, infused iv at 25 µL/min during a 7 min period,), and PHE (continuous iv infusion at 7 μg/kg/min dissolved in 10µL 0.9% NaCl) (See Fig. 5).

Selection of drug doses for in vivo experiments.

Drug doses for in vivo experiments were based on earlier work by other groups (e.g. 8). For ET-1, a dose-finding study was performed (n = 4). In anaesthetized rats, ET-1 did not cause pressor responses at dosages below 1 µg/kg. Instead, a transient depressor response was observed (data not shown). At 1 µg/kg, ET-1 caused small but long-lasting pressor responses. Several animals receiving ET-1 at 3 µg/kg died within 20 min. most likely due to organ failure from severe blood flow limitation. Hence, we chose 1 µg/kg for further studies. Big-ET-1 and ET-1 are equipotent with regards to inducing pressor responses9. Therefore we used 1 µg/kg big-ET-1. In conscious rats, 1 µg/kg of ET-1 did not raise blood pressure. Therefore, we performed an additional dose-finding study. In this study we found that short term infusion of ET-1 (2.2 µg/kg infused during 7 min) induced long-lasting pressor responses. The dose of CGRP was based on the literature8. The dose of SNP was chosen to evoke a similar, or more pronounced reduction of RR, MR, HQR and MAP.

Drugs and solutions.

CAPS10,11 was purchased from Sigma Aldrich (Zwijndrecht, NL) and dissolved in ethanol. SNP, and PHE were purchased from Sigma Aldrich (Zwijndrecht, NL) and dissolved in KRB solution. BQ1235 and BQ7886 were obtained from Bachem (Weil am Rhein, D) and dissolved in DMSO. Human αCGRP, ET-1, big-ET-1 and sarafotoxin 6c were obtained from Bachem (Weil am Rhein, D) and dissolved in KRB solution. The maximal concentrations of the solvents never exceeded 0.1 % and did not alter arterial reactivity.
Supplemental references


Table S1: General arterial characteristics and characteristics of ET-1-induced contractions.
Diameter was assessed at 0.9D_{100}. K^{+}_{\text{max}} represents the maximal contractile response to 40 mM K^{+}. EC_{50} of ET-1 is defined as the concentration at which 50% of the maximal response to the peptide develops. E_{\text{max}} ET-1 is defined as the maximal contractile response to 32 nM ET-1. n = 6 – 9.

<table>
<thead>
<tr>
<th>Artery</th>
<th>Diameter (μm)</th>
<th>K^{+}_{\text{max}} (N/m)</th>
<th>EC_{50} ET-1 (nM)</th>
<th>E_{\text{max}} ET-1 (% K^{+}_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basilar artery</td>
<td>173 ± 19</td>
<td>0.77 ± 0.11</td>
<td>6.4 ± 0.3</td>
<td>152 ± 34</td>
</tr>
<tr>
<td>Coronary artery</td>
<td>171 ± 11</td>
<td>0.27 ± 0.10</td>
<td>9.6 ± 0.2</td>
<td>350 ± 79</td>
</tr>
<tr>
<td>Epigastric artery</td>
<td>150 ± 16</td>
<td>0.92 ± 0.15</td>
<td>6.7 ± 0.2</td>
<td>357 ± 66</td>
</tr>
<tr>
<td>Gastric artery</td>
<td>284 ± 16</td>
<td>1.56 ± 0.38</td>
<td>76.7 ± 2.4</td>
<td>145 ± 23</td>
</tr>
<tr>
<td>Mestenteric artery</td>
<td>275 ± 12</td>
<td>2.34 ± 0.45</td>
<td>5.8 ± 0.1</td>
<td>128 ± 9</td>
</tr>
<tr>
<td>Renal artery</td>
<td>228 ± 19</td>
<td>1.29 ± 0.31</td>
<td>9.4 ± 0.1</td>
<td>239 ± 61</td>
</tr>
<tr>
<td>Saphenous artery</td>
<td>217 ± 17</td>
<td>2.50 ± 0.42</td>
<td>15.5 ± 0.3</td>
<td>152 ± 13</td>
</tr>
<tr>
<td>Spermatic artery</td>
<td>198 ± 7</td>
<td>0.79 ± 0.24</td>
<td>12.1 ± 0.1</td>
<td>200 ± 15</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>180 ± 17</td>
<td>1.19 ± 0.36</td>
<td>9.3 ± 0.2</td>
<td>395 ± 118</td>
</tr>
</tbody>
</table>
Figure S1: ET\textsubscript{B} receptor activation does not contract isolated arteries. Exposure of freshly isolated rat basilar, coronary, epigastric, gastric, mesenteric, renal, saphenous, spermatic or splenic arteries to the ET\textsubscript{B} receptor selective agonist sarafotoxin 6c (1 – 128 nmole/L) does not cause vasoconstriction. Data are expressed as % of K\textsuperscript{+}\textsubscript{max} and are shown as mean ± SEM (n = 3)